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UNIVERSITY OF ALBERTA

DYNAMICS OF MICROBIAL BIOMASS, SOIL FAUNA AND NITROGEN IN CONTRASTING SOILS

BY

PERRY MICHAEL RUTHERFORD

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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Of

DOCTOR OF PHILOSOPHY

IN

SOIL MICROBIOLOGY AND BIOCHEMISTRY

DEPARTMENT OF SOIL SCIENCE

EDMONTON, ALBERTA

FALL 1991

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Department of Soil Science

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Associate Professor

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Abstract

The objective of this study was to link dynamics of soil organisms with N cycling in some arable soils from central Alberta. In soils fertilized with ^{15}N urea and cropped to barley, microbial C (g m^{-2}), nematode, acari and collembola populations (no. m^{-2}) were greater in a Black Chernozemic soil at Ellerslie than in a Gray Luvisolic soil at Breton. On average, shoot N and shoot ^{15}N recoveries were greater at Ellerslie than at Breton. Total ^{15}N recovery was similar between sites but soil ^{15}N was greater at Breton. Ten-day laboratory incubation of 0-10 cm soil samples from Ellerslie evolved more CO_2-C and mineralized more N (μg g^{-1} soil) compared to samples from Breton. Microbial C and protozoa numbers expressed per unit of soil C were greater in the Gray Luvisol, and acari and collembola populations were not significantly different. CO_2-C evolution (mg C g^{-1} C soil) and net N mineralized (μg N g^{-1} N soil) were greater in the Gray Luvisol than in the Black Chernozem. A larger proportion of the soil organic matter in the Gray Luvisol was kinetically active and more available to the biological community than in the Black Chernozem. Glucose addition with fertilizer N application increased microbial activity and reduced losses of fertilizer N by increased immobilization. Remineralization of N was not sufficient to meet the demands of the barley crop.

In a microcosm experiment using three Orthic Black Chernozem Ap samples, an *Acanthamoeba* reduced *Pseudomonas* numbers and increased the mineralization of bacterial ^{15}N more in a sandy loam than in a silty clay and a clay loam. Bacteria were more protected from protozoan predation in the fine-textured soils than in the coarse-textured soil. A model was developed to simulate population dynamics and flows of C, ^{14}C, N and ^{15}N. The model showed that predation affected the partitioning of immobilized N depending on the texture of the soil. Protozoa mineralized twice as much NH_4-N as did bacteria during the first 12 days, thereby increasing NH_4-N three-fold compared to the protozoa-minus treatment. The link between soil biota and N cycling was developed through simulation modelling.

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Chapter 1. Introduction

The five kingdoms of organisms (Monera, Fungi, Protista, Animalia, Plantae) are represented in soil (Price 1988). The microbial component consists mainly of bacteria, actinomycetes, fungi, small algae and single-celled protozoa. Common mesofauna and macrofauna in arable soils also include nematodes (Nematoda), springtails (Collembola), mites (Acari), potworms (Enchytraeidae) and sometimes earthworms (Lumbricidae). In natural grassland and forest soils other fauna such as Gastropoda, Crustacea, Myriapoda, Insecta and Arachnida may also be common. Compared to higher plants, soil organisms form only a small part of the total biomass in terrestrial ecosystems but they are of prime importance in elemental cycling and in the flow of energy (Richards 1974). The numerous roles of soil organisms have been described (Russell 1961, Wallwork 1970, Gray and Williams 1971, Alexander 1977, Lohm and Persson 1977, Hole 1981, Coleman et al. 1983, Tinsley and Darbyshire 1984, Mitchell and Nakas 1986, Edwards et al. 1988, Paul and Clark 1989). Root inputs and litter inputs from higher plants provide most of the energy and carbon inputs for the free-living heterotrophs of the decomposer food web. Since most basic links in terrestrial food webs exist in the soil, all efforts to achieve a comprehensive understanding of trophic systems should begin and end with the soil biota (Price 1988).

One of the major tasks in ecology is to characterize ecological systems and to relate structural characteristics to functional processes (Richards 1974). Most work in ecology has focused on macroscopic organisms, but microscopic soil organisms occupy a much broader range of niche spaces, and carry out more complex ecological interactions, than larger organisms (Price 1988). Though the study of soil organisms began in the late 1800's with the birth of soil microbiology (Russell

1961), the science of soil biology has only been fully appreciated as a scientific endeavor for the past 40-50 years (Edwards and Stinner 1988). Early studies were descriptive and often considered organisms in isolation. Current studies are more integrative and attempt to unravel the interactions between trophic groups and their environment, and how activities of the soil biota impact on soil processes.

It is challenging to assess the structure of soil food webs because enumeration of a wide range of organisms is difficult and time consuming. In the past soil microbiologists and soil zoologists have often worked independently (Edwards and Stinner 1988), but it is important to study simultaneously as many groups of organisms as possible to obtain an overall understanding of the soil system function (Hunt et al. 1987). One approach to reducing the complexity of the system is to place various taxa into broad functional groups thereby negating the requirement of considering the activities of each species separately.

Much work in soil biology is from grassland soils and forest soils (Petersen and Luxton 1982). In the past 10 years, research in soil ecology has intensified in arable soils (Elliott et al. 1984a, Hendrix et al. 1986, Brussaard et al. 1988, Clarholm and Bergström 1989, Andrén et al. 1990). Though certain basic principles apply in all soils, management practices can alter food web structure and function (Hendrix et al. 1986). Therefore further work in arable soils is warranted.

Soils form at the interface of the lithosphere, biosphere, hydrosphere and atmosphere. The factors of soil formation (parent material, climate, topography, biota (mainly plants), time) control soil development processes (additions, transformations, translocations, losses) to determine the properties, and hence the types of the soils which develop (Simonson 1959). Soil chemical and physical properties are expected to influence, and be influenced by the soil biota. However, agronomists, soil physicists, soil chemists and pedologists have historically not worked jointly with soil biologists (Kevan 1985). There is a need to bridge the gap between soil biology and these traditional areas of soil science by means of more integrative research studies.

Nutrient cycling, especially nitrogen cycling, is one of the important indexes of ecosystem function. Nitrogen (N) is often the most limiting nutrient to plant growth (Brady 1974). A large proportion of soil N exists in organic forms (Stevenson 1986) and is unavailable for plant use until it is first converted to NH_4-N by the process of net N mineralization. The activities of the soil bacteria and fungi, in their search for energy and carbon (C), are largely responsible for the mineralization-immobilization turnover of soil N (Jansson 1982). Even though soil fauna are responsible for less than 10% of soil biological activity (Peterson and Luxton 1982) they play an important role by regulating the size and activity of the microbial biomass (Elliott et al. 1984b) and in C and N mineralization (Coleman et al. 1983).

Knowledge of soil organic C and N is necessary to assess ecosystem structure and function. Soil organic C and N have been studied by four main approaches over the past 100 years: (i) acid hydrolysis (ii) classical humic fractionation techniques (iii) particle size fractionation, and (iv) the kinetic approach, which categorizes heterogeneic organic materials by their decomposition rates and disregards their chemical identity (Juma and McGill 1986). The kinetic approach, developed by Paul and Juma (1981), furthers the pioneering work of Jansson (1958) and Jenkinson (1966) by dividing soil organic N into four pools: (i) microbial biomass (mainly bacteria and fungi) (ii) non-microbial active N (iii) stabilized fraction, and (iv) old organic matter. This approach has been successful at predicting mineralization-immobilization turnover of N in soils (Juma and McGill 1986). The advantage of this method of organic matter study is that Jenkinson's chloroform fumigation method for determination of microbial C and N can be used to directly link the decomposer food web with elemental cycling. Use of ^{14}C and ^{15}N tracers allows the dynamics and partitioning of amended C and N to be followed amongst various pools in the plant-soil system. Population dynamics of microflora and fauna provide further insight into the role of microbial-faunal interactions in determining C and N dynamics in various soils. Further research linking organic matter dynamics and N cycling to the soil food web, and if manipulations of the food web is required.

Simulation models are useful tools in ecological research because they improve our understanding of systems, rather than serving as predictive devices (van Veen et al. 1981). Models reduce ambiguity when describing complex systems and they provide a framework for the integration and testing of existing information and of hypotheses (Jeffers 1982). In addition, their construction can help identify gaps in knowledge and are useful to identify critical experiments (Jeffers 1982). Modelling has greatly improved our understanding of soil organic matter dynamics and nutrient cycling (Juma and Paul 1981, McGill et al. 1981, Voroney et al. 1981, van Veen et al. 1984, Parton et al. 1988) and of food web interactions in soils (Hunt et al. 1984, 1987, 1989; Moore et al. 1990). Further modelling is needed to aid in our understanding of the linkage between food web dynamics and nutrient cycling in contrasting soils.

The overall objective of this study was to provide a greater understanding of the role of soil organisms on N dynamics in some Canadian arable soils. Black Chernozemic and Gray Luvisolic soils are used extensively in Canada for agricultural production (Acton 1978) and they represent extremes in terms of agricultural productivity (Bentley et al. 1971, Dinwoodie 1988). More specific objectives were (i) to compare field populations of soil organisms present in a Black Chernozemic soil and a Gray Luvisolic soil from central Alberta, and to compare the overall activity of the organisms in the two soils; (ii) to determine if N cycling differs in the two soils, and to determine if the sizes and activities of the active N pools are different and if this relates to the activity of the organisms; (iii) to determine if glucose addition affects the recovery and partitioning of added urea-N among various pools in the plant-soil system and to evaluate whether there is a long-term effect of glucose addition on biological activity; (iv) to determine if texture affects habitable pore space in soil and if this affects the protection of bacteria from protozoan predation; (v) to determine if texture affects the ability of protozoa to stimulate mineralization of bacterial C and N; and (vi) to construct a simulation model that incorporates information about organic matter dynamics and food web interactions to explain the data generated in (iv) and (v). Chapter 8 provided a synthesis of the thesis, with some overall conclusions.

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Chapter 2. Dynamics of Microbial Biomass and Soil Fauna in Two Contrasting Soils Cropped to Barley*

Introduction

The population size and activity of members of the soil biota in agroecosystems are controlled by management factors such as cropping system (Sohlenius et al. 1987), tillage (House and Parmelee 1985, Parmelee and Alston 1986, Foissner 1987), amendments such as fertilizers (Sohlenius and Boström 1986), interactions between organisms (Coleman 1985), environmental conditions such as temperature and water regimes (Yeates 1981, Moorhead et al. 1987) and soil type (Andrén and Lagerlöf 1983, Hendrix et al. 1986). The response of soil biota to changes in management or environmental conditions is often accomplished by studying the dynamics of organisms over the growing season (Campbell and Biederbeck 1976, Freckman et al. 1987). However research in agroecosystem ecology is difficult to interpret due to the large number of interactions involved. Examination of interactions between microbial biomass, protozoa, nematodes, acari and collembola in arable soils is incomplete (Elliott et al. 1984a). Consequently, there is a need to examine the dynamics of as many members of the soil biota as is possible.

Community-level investigations provide a context to define the role of individual members and to discover synergistic effects (Hunt et al. 1987). Soil biota modulate the flow of energy, carbon and nutrients in soil (Elliott et al. 1984b). Soil microbial biomass is a major source, sink and transformation agent of plant nutrients (Anderson and Domsch 1980, Paul and Voroney 1980) and is directly responsible for >90% of soil respiration (Petersen and Luxton 1982). Protozoa and nematodes are important regulators of the activity of microbial biomass (Bamforth

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1985, Clarholm 1984, Coleman 1985) and accelerate mineralization of C, N and P in the rhizosphere and may increase availability of nutrients to crops (Elliott et al. 1984b, Clarholm 1985, Ingham et al. 1985). Acari and collembola are mainly detritus and fungal feeders and accelerate decomposition of C and mineralization of plant nutrients (Seastedt 1984). The availability of C governs the size and composition of the biological community and is controlled by texture (van Veen et al. 1985), mineralogy (Pawluk 1986) and chemical composition and amount of organic matter (Stewart 1987).

Soils belonging to the Chernozemic and Luvisolic orders are important for cereal production in Alberta and represent two extremes of natural fertility; biological, physical and chemical properties; and crop productivity. The objectives of the present study were to: (i) compare under field conditions the dynamics of shoots, roots, microbial biomass and faunal populations in two pedogenically different soils cropped to barley; and (ii) compare under laboratory conditions the C flux, microbial and faunal activity in these soils. The two soils used were a Black Chernozem and a Gray Luvisol. The work was part of a larger integrative study which also investigated C and N dynamics at the two sites.

Materials and Methods

Site Description and Soils: This study was conducted at the Ellerslie Research Station and the Breton Research Plots. The University of Alberta Ellerslie Research Station is approximately 10 km south of Edmonton, Alberta (53 25' N, 113 33' W) on a Black Chernozemic soil (Malmo series). The plots at this site, which were in hay production between 1971 and 1984, were plowed in 1984 and seeded to barley in 1985. The University of Alberta Breton Research Plots are located near the town

Table II.1. Soil Properties at Ellerslie and Breton

Depth (cm)	Total C (%)	Total N (%)	pH (1:2 soil:H_2O) (mass:volume)	Bulk Density (Mg m^{-3})	Texture
		Ellerslie (Black Chernozem)			
0-10	6.46	0.534	6.1	0.86	SiCL
10-20	6.32	0.491	6.0	1.06	
20-30	5.23	0.419	6.0	1.17	
		Breton (Gray Luvisol)			
0-10	2.17	0.185	6.2	1.11	SiL
10-20	1.92	0.164	6.3	1.30	
20-30	1.13	0.104	6.1	1.55	

of Breton, Alberta (53 07' N, 114 28' W), approximately 110 km SW of Edmonton. The soil is a Gray Luvisol (Breton series). The plots used had been cropped to barley since 1981. Soil properties for both sites are presented in Table II.1.

Average annual precipitation at Ellerslie is 452 mm, of which 339 mm occurs as rain and 113 mm as snow. Breton receives 547 mm of precipitation annually, of which 405 mm is rain and 132 mm is snow. Both sites receive the greatest rainfall in June, July and August, and the greatest snowfall in December and January. July is the warmest month at both sites with an average minimum temperature of 9.6 °C at Ellerslie and 8.8 °C at Breton, and a maximum of 22.4 °C at Ellerslie and 21.2 °C at Breton. January is the coldest month with average minimum temperatures of -21.7 °C at Ellerslie and -19.5 °C at Breton, and average maximum temperatures of -11.5 °C at Ellerslie and -8.6 °C at Breton. Ellerslie has an average of 109 frost free days per year and Breton an average of 80.

Experimental Design and Sampling Schedule: The experiment was a factorial split-plot design with three blocks at two sites. Four open-ended cylinders (20 cm internal diameter, 30 cm long) were pressed into each block in late May 1986. On May 28 1986 the cylinders were seeded (8 seeds per cylinder) to barley (*Hordeum vulgare* L., cv. Empress) and fertilized with 10 mL of urea solution (23 mg N mL^{-1}; 75 kg N ha^{-1}). The area surrounding the cylinders was also seeded to barley and fertilized with urea (77 kg N ha^{-1}) and triple superphosphate (47 kg P ha^{-1}) three days prior to the installation of the cylinders. No herbicides were used and the few weeds that appeared were included in the plant analyses.

At each sampling date the shoot material from a cylinder in each block was cut at ground level. Cylinders for each sampling date were excavated from each block and soil was divided into three depths: 0-10, 10-20 and 20-30 cm in the laboratory. Roots were separated from soil by hand. Soil samples were stored moist in plastic bags at 5°C. At Ellerslie the plots were sampled on July 31, August 18, September 8 and September 29, which corresponded to 64, 82, 103 and 124 days after sowing, respectively. At Breton the plots were sampled on August 11, September 1, September 22 and October 20, which corresponded to 75, 96, 117 and 145 days after sowing. Early season samples were not taken due to wet conditions and laboratory renovations. The last Breton sampling date was also delayed because of wet field conditions.

Analyses: Shoot and root material was dried at 70°C and weighed. Soil mass and gravimetric water contents were determined on unsieved soil from each depth interval. Water-filled porosity (%) was calculated using the equation:

$$\%WFP = (\theta m \times Db/(1-(Db/Dp)) \times 100$$ (Linn and Doran 1984),

where θm= gravimetric water content, Db and Dp are bulk and particle density, respectively. Bulk density (Db) was calculated from the mass of oven dry soil from each layer divided by the volume occupied in the cylinder. Particle density (Dp) was assumed to be 2.65 Mg m^{-3}.

All biological measurements were performed within two days after sampling. The soil from each depth was uniformly mixed prior to each analysis. Field moist unsieved soil was used for the enumeration of protozoa, nematode and microarthropod populations. Microbial C measurements were performed on sieved (10 mesh) soil adjusted to 55% water holding capacity.

Microbial respiration and microbial C were measured on duplicate 25-g moist, sieved soil samples by the chloroform fumigation method (Jenkinson and Powlson 1976). Microbial C (B_c) was calculated from the formula $B_c = F_c/K_c$, where F_c is {(C mineralized in fumigated soil incubated for 10 days)-(C mineralized from non-fumigated control incubated for 10 days)} and $K_c = 0.411$ (Anderson and Domsch 1978). The CO_2-C evolved from non-fumigated samples was used as a measure of soil respiration. The flush of mineral N following fumigation and incubation {(mineral N (NH_4-N + NO_3-N) in fumigated soil incubated for 10 days)-(mineral N in non-fumigated soil incubated for 10 days)} was determined on soil extract samples using Technicon Auto Analyzers (Chapter 3).

Protozoa were determined in duplicate for each of the 0-10 and 10-20 cm soil samples by the most probable number technique (MPN). Field moist soil (20 g) was added to 100 mL of 3% soil extract and serially diluted to 10^{-8}. Eight tubes were made per dilution. Dilution tubes were incubated at 22°C and scored positive or negative after one week by microscopic examination of three drops of culture in a microtitre dish. Negative tubes were checked approximately two weeks later for positive growth. MPN were calculated by using a microcomputer program developed by Clarke and Owens (1983).

Nematodes were extracted in triplicate for each of the 0-10 and 10-20 cm soil samples by using a combination Cobb wet sieving and modified Baermann funnel procedure (Van Gundy 1982). Wet sieving concentrated nematodes from 400 g soil into a subsample of approximately 20 g. This soil concentrate was placed on a double layer of tissue paper resting in a 15 cm petri dish. The sample was saturated with tap water for two days at 20°C. The nematodes were then subsampled from the tap water and counted using a stereo microscope.

Microarthropods were extracted from six 50-g replicates of each sample for six days using a high gradient heat extractor (Berg and Pawluk 1984). The microarthropods were collected in ethylene glycol and were transferred to small vials with ethanol for separation into acari and collembola. No further taxonomic classification was undertaken in this study.

Statistical analyses: Analysis of variance was performed on all experimental variables. Preliminary analysis determined that faunal counts were non-normal with heterogeneous variance. A log10 transformation was performed on nematode, protozoa and microarthropod data. Selected experimental parameters were sorted by site and depth and tested for relationships by Pearson correlation matrices. All reported correlations are significant at $p \leq 0.05$. All results reported in the figures are averages of three replicates.

Results

Weather and Soil Physical Conditions

Mean air temperature did not differ between sites and ranged from 12 to 16°C during June to late August, 1986. Mean air temperature dropped to 6°C between late August and mid-September and increased to 9°C in October. Both sites were wet, however Breton received approximately 285 mm in July, almost three times its 30-year mean of 95 mm (Figure II.1). Ellerslie received 126 mm in July, approximately 1.5 times the 30-year mean of 85 mm. High precipitation at Breton combined with slow infiltration into the soil resulted in ponded water over the cylinders in July. The first sampling date was delayed until early August when saturated conditions ceased.

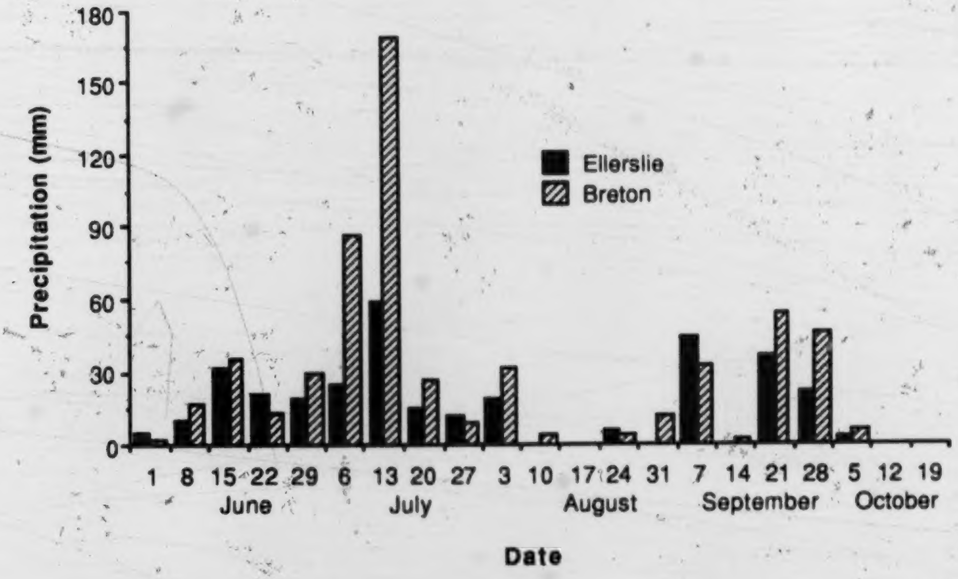

Figure II.1. Weekly Precipitation at Ellerslie and Breton in 1986

Bulk densities (Tables II.1 and II.2) were greater at Breton and increased with depth more at Breton than at Ellerslie. A hard compacted layer was observed in the Breton soil at approximately 15 cm from the surface. There was no difference in gravimetric soil water content between sites (Fig. II.2 and Table II.2). The water content at Ellerslie dropped more in August (date 2) and early September (date 3) than at Breton. Gravimetric water contents ≤18% and ≤11% corresponded to soil water potentials ≤-1.5 MPa in the surface horizons of the Black Chernozem and Gray Luvisol, respectively. The soil surface layers were wetter than subsurface layers due to precipitation for dates one and four in contrast to the drying curves for dates two and three. Water-filled porosity (WFP) was greater at Breton due to its lower total porosity (Fig. II.2 and Table II.2). This was very pronounced in the 20-30 cm depth where WFP was probably >80% all summer. WFP did not decrease as much in the mid-summer at Breton compared to Ellerslie. Unlike gravimetric water content, WFP was almost always greater for increasing depths. Water-filled porosity was up to 2.2-fold greater in the 20-30 cm depth at Breton compared to Ellerslie.

Table II.2. Summary of ANOVA[a] for Bulk Density, Soil Water and % Water-Filled Porosity at Ellerslie and Breton

Source of Variation	Bulk Density	Soil Water	Soil WFP
Site (S)	***	ns	*
Date (D)	ns	***	***
S x D	ns	***	*
Depth (Dp)	***	ns	***
S x Dp	†	ns	*
D x Dp	ns	***	ns
S x D x Dp	ns	*	ns

In this and all subsequent tables:

[a] The difference between means is significant at: †, $p \leq 0.10$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$

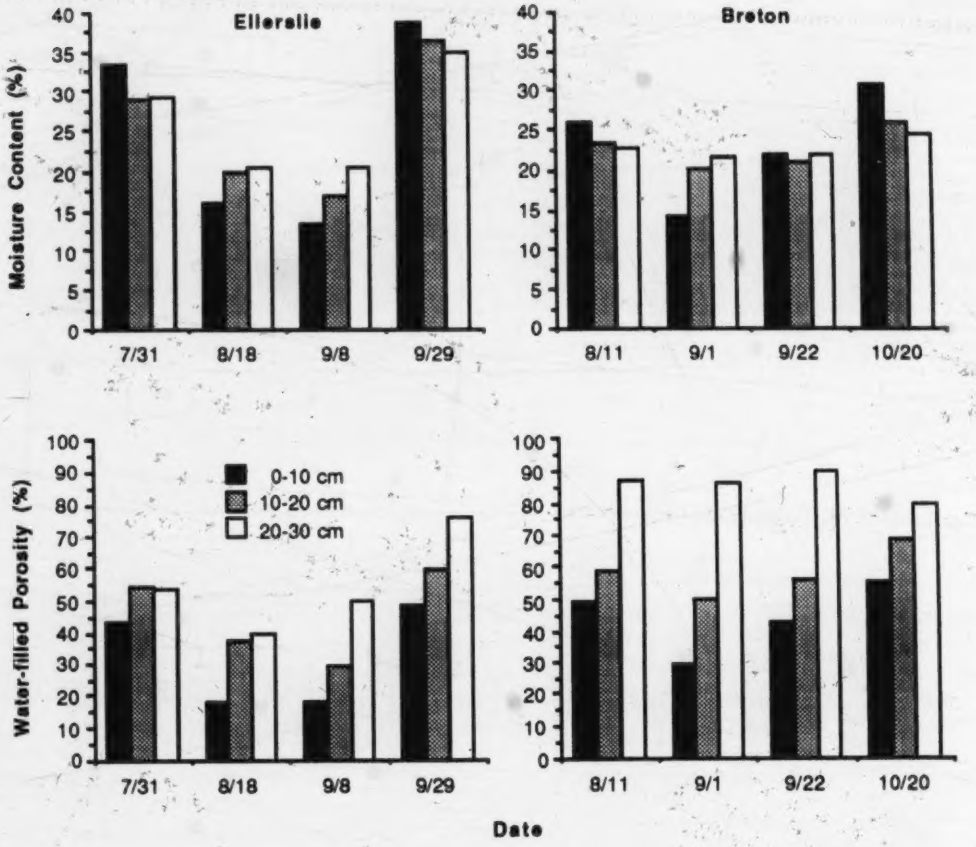

Figure II.2. Gravimetric Water Content and Water-filled Porosity in Soil from Ellerslie and Breton

Shoot and Root Mass

On average shoot mass was 1.5-fold greater at Ellerslie compared to Breton (Table II.1). Shoot mass decreased over the sampling period at Breton but peaked on the third sampling date at Ellerslie. Root mass did not differ between sites or dates but decreased with depth. A larger proportion of roots were in the 0-10 cm interval at Breton compared to Ellerslie.

Microbial Biomass

On average microbial C was 1.9-fold greater at Ellerslie compared to Breton (Figure II.3 and Table II.4). At both sites the microbial C did not change substantially with time, however it decreased with depth.

The ratio of the flush of CO_2-C/ flush of mineral N (NH_4-N + NO_3-N) over the 10-day incubation following fumigation (flush C/N ratio) (Figure II.3 and Table II.4) was greater at Ellerslie compared to Breton. Flush C/N ratio averaged 6.3 at Ellerslie and 4.7 at Breton for the entire 0-30 cm interval. The flush C/N ratio was 1.3-fold greater in the 0-10 cm interval at Ellerslie compared to Breton ($p \leq 0.05$). Flush C/N ratio at Breton was lowest on the second sampling date. At Ellerslie it was high for the second and third sampling dates compared to the first and fourth dates. At both sites the flush of N decreased with depth more than the flush of C resulting in a significantly greater flush C/N ratio with depth. This was most pronounced in the 20-30 cm depth.

Table II.3. Shoot Mass and Root Mass at Ellerslie and Breton (means of 3 replicates)

Variable	Depth (cm)	Days After Sowing[a]			
		64/75	82/96	103/117	124/145
		Ellerslie			
Shoot Mass (g m^{-2})		540	561	878	650
Root Mass (g m^{-2})	0 - 10	111	41	45	62
	10 - 20	17	11	17	13
	20 - 30	8	5	7	8
Shoot Mass to Root Mass		3.9	9.8	12.6	8.1
		Breton			
Shoot Mass (g m^{-2})		582	450	419	279
Root Mass (g m^{-2})	0 - 10	165	73	62	54
	10 - 20	9	5	6	7
	20 - 30	1	2	1	1
Shoot Mass to Root Mass		9.1	5.8	6.6	4.8

Summary of ANOVA

Source of Variation	Shoot Mass (g m^{-2})	Root Mass (g m^{-2})	$\frac{\text{Shoot Mass}}{\text{Root Mass}}$
Site (S)	†	ns	ns
Date (D)	ns	ns	ns
S x D	*	ns	*
Depth (Dp)		***	
S x Dp		ns	
D x Dp		†	
S x D x Dp		*	

[a] Sampling date for Ellerslie and Breton respectively

Table II.4. Summary of ANOVA for Microbial C, Flush C/N Ratio and Soil Fauna

Source of Variation	Microbial C	Flush C/N Ratio	Protozoa^a	Nematodes^b	Acari^b	Collembola^c
Site (S)	**	***	ns	*	†	†
Date (D)	ns	***	ns	*	*	ns
S x D	ns	***	**	ns	ns	**
Depth (Dp)	*	***	†	**		
S x Dp	ns	*	ns	ns		
D x Dp	ns	ns	*	ns		
S x D x Dp	ns	ns	ns	ns		

[a] Determined on transformed MPN (log10 (no. m^{-2}))

[b] Determined on transformed counts (log10 (no. m^{-2}))

[c] Determined on transformed counts (log10 (x+1)) before convertion to m^2 basis since zero counts were obtained

At Ellerslie, flush C/N ratios at 0-10 and 10-20 cm were negatively correlated with soil water (r=-0.590 and -0.565, respectively) and WFP (r=-0.650 and -0.606, respectively). This relationship was not found in the 20-30 cm depth at Ellerslie or at any depth at Breton.

Faunal Populations

Protozoa populations were variable yet some significant differences were observed (Figure II.4 and Table II.4). Populations in the 0-20 cm depth tended to decrease with time at Ellerslie but increase with time at Breton. Overall, populations were slightly greater in the 0-10 cm depth compared to the 10-20 cm depth. On date four, high populations in the 10-20 cm depth at Ellerslie and in the 0-10 cm depth at Breton contributed to a significant date by depth effect.

Nematode populations were 5-fold greater at Ellerslie compared to Breton. At both sites the sum of populations at both depths increased throughout the sampling period. Nematode populations decreased with depth at both sites.

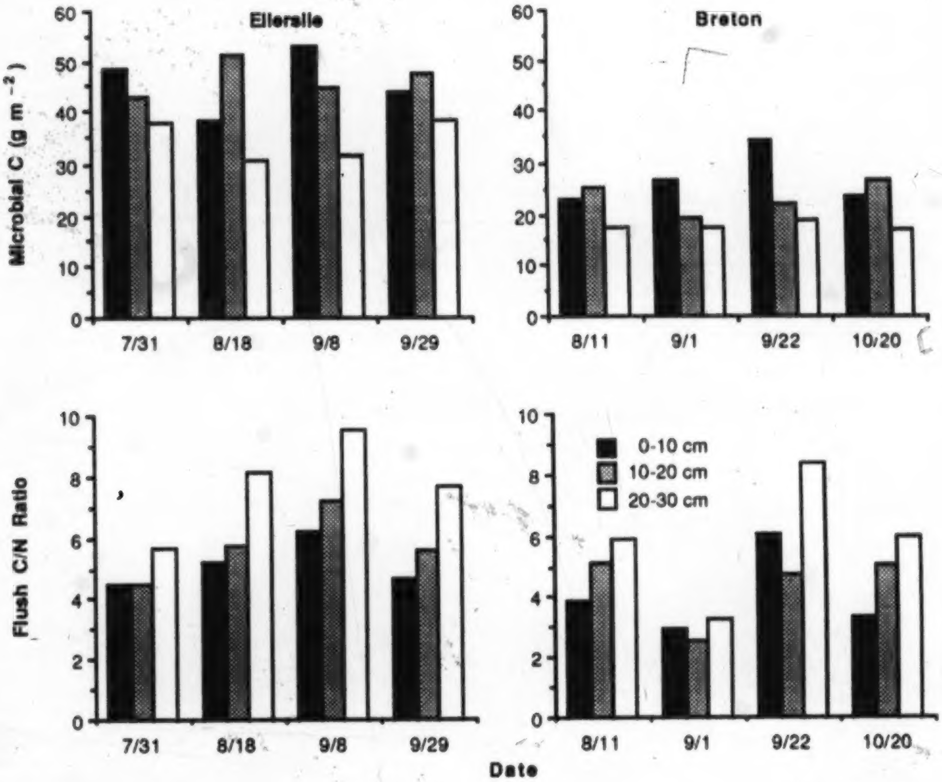

Figure II.3. Microbial C and Ratio of the Flush of CO_2-C to the Flush of Mineral N (NH_4-N + NO_3-N) Over the 10-day Incubation Following Fumigation

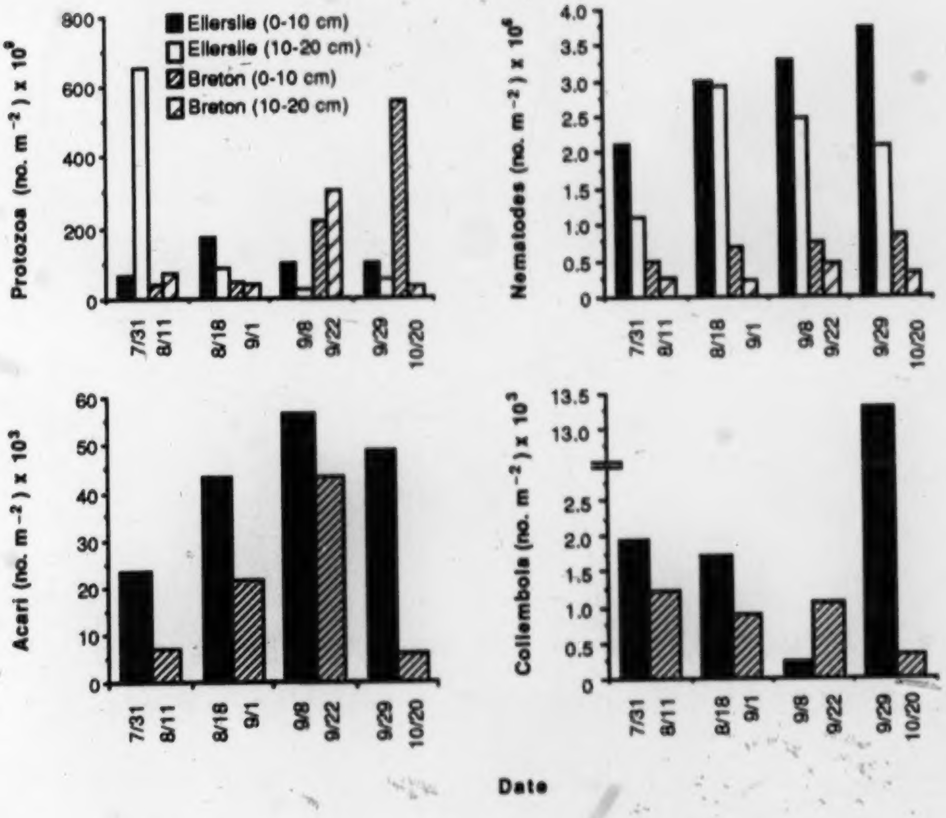

Figure II.4. Soil Fauna at Ellerslie and Breton

On average, acari populations (Figure II.4 and Table II.4) were 2.2-fold greater at Ellerslie. Populations peaked at the third sampling date at both sites. At Breton acari populations dropped off sharply on the fourth sampling date.

On average, collembola populations (Figure II.4 and Table II.4) were 4.8-fold greater at Ellerslie compared to Breton. However populations differed greatly on date four at the two sites. At Ellerslie the population mean increased from 230 (no. m^{-2}) on date three to 13200 (no. m^{-2}) on date four. Populations at Breton decreased from 1100 (no. m^{-2}) on date three to 320 (no. m^{-2}) on date four. Collembola were positively correlated with soil water content at Ellerslie ($r=0.580$).

Absolute Versus Relative Biological Size and Activity in the Surface Interval at Ellerslie and Breton

Although microbial C (g m^{-2}) was greater at Ellerslie, microbial C made up a larger proportion of soil C (mg g^{-1} soil C) at Breton (Table II.5). Protozoa (no. m^{-2}) were not significantly different between sites but were greater at Breton when expressed relative to soil C (no. g^{-1} soil C). Acari, collembola and nematodes were greater at Ellerslie when expressed on an area basis (no. m^{-2}) however did not significantly differ between sites when expressed relative to soil C (no. g^{-1} soil C). Soil C at Breton supported proportionally greater microbial C and protozoan populations and equal nematode and microarthropod populations compared to Ellerslie (Table II.5).

Ten-day incubations of non-fumigated soil showed that CO_2-C evolved was greater at Ellerslie (120 μg g^{-1} soil) than Breton (97 μg g^{-1} soil) (data not shown), however there was no significant difference in CO_2-C evolution when expressed on an area basis (g m^{-2}) between soils (Table II.5). CO_2-C evolution was greater from

Table II.5. Absolute and Relative Comparisons of Selected Variables in 0-10 cm Depth at the Two Sites (means of 12 replicates)

	Site		Significance
Variable	Ellerslie	Breton	of Site Effect
	Absolute (per m^2 basis)		
Soil C ($g\ m^{-2}$)	5529	2376	***
Microbial C ($g\ m^{-2}$)	45.9	26.4	*
Protozoa (no. m^{-2}) x 10^9	108	211	ns
Nematodes (no. m^{-2}) x 10^5	30.2	6.9	**
Acari (no. m^{-2}) x 10^3	42.7	19.7	†
Collembola (no. m^{-2}) x10^2	43	9	†
CO_2-C Evolved ($g\ m^{-2}$ 10 $days^{-1}$)	10.3	10.7	ns
	Relative (per g soil C or microbial C)		
Microbial C (mg g^{-1} soil C)	8.2	11.1	*
Protozoa (no. g^{-1} soil C) x 10^6	19	88	*
Nematodes (no. g^{-1} soil C) x 10^2	5.5	2.9	ns
Acari (no. g^{-1} soil C)	7.7	8.3	ns
Collembola (no. g^{-1} soil C)	0.80	0.36	ns
CO_2-C Evolved (mg g^{-1} soil C 10 $days^{-1}$)	1.9	4.5	***
CO_2-C Evolved (g g^{-1} microbial C 10 $days^{-1}$)	0.23	0.41	**

Breton soil samples when expressed as a proportion of soil C (mg g^{-1} soil C) or as a proportion of microbial C (mg g^{-1} microbial C) (Table II.5).

Discussion

Plant, Microbial, and Faunal Interactions in Two Pedogenetically Different Soils

The major environmental factor influencing biological activity was the unusually high precipitation during early summer at Breton (Figure II.1). Although mean gravimetric water content over the sampling period was not statistically different between sites, the soil from Breton had significantly greater water-filled porosity (WFP) (Figure II.2). According to Linn and Doran (1984) anaerobic activities such as denitrification become prevalent when WFP exceeds 70%. WFP exceeded 70% in the 20-30 cm depth at Breton throughout the sampling period. Prior to the first sampling date the entire 0-30 cm interval probably had >70% WFP for a few days or weeks. Therefore the physical environment differed greatly at the two sites.

Wet conditions at Breton affected barley growth. On average, shoot N concentration was significantly lower at Breton (1.1%) than Ellerslie (2.1%). Lower shoot C/root C ratios (Dinwoodie and Juma 1988) at Breton indicated N deficient conditions (Hansson et al. 1987) and/or loss of leaves (Sallam and Scott 1987). Greater shoot mass at Ellerslie was due to the combination of greater natural fertility and more favorable environmental conditions at that site compared to Breton. The decline of shoot mass at Breton was likely due to waterlogging which caused premature senescence. Shoot mass at Ellerslie did not change much over the sampling period because plants were almost mature by the first sampling date. Peak shoot mass at Ellerslie (878 g m^{-2}) was similar to those reported by Hansson and Steen (1984) and Hansson et al. (1987) (942 and 840 g m^{-2}, respectively) but was lower at Breton (582 g m^{-2}).

There was no difference in mean root mass between sites, however the plants at Breton had 89% of their total root mass in the 0-10 cm depth compared to 74% at Ellerslie. Several factors probably contributed to the differences in root distribution. Bulk density and WFP at Ellerslie were much lower in the 10-30 cm interval than at Breton resulting in easier root penetration and greater aeration. The compact layer observed at approximately 15 cm in the Breton soil probably restricted root penetration. The soil at Ellerslie also had a deeper A horizon (>30 cm) than at Breton (~17 cm) resulting in a more gradual decline of total N, organic C and exchangeable bases. Peak root mass (g m^{-2}) at Ellerslie (135) and Breton (79) were greater than those reported by Hansson and Steen (1984) (60 g m^{-2}).

Microbial C and nematode, acari and collembola populations were greater at Ellerslie compared to Breton. This was expected since the Black Chernozemic soil at Ellerslie had a much greater total organic C content. Microbial biomass (Schnürer et al. 1985, Carter 1986) and faunal populations (Norton 1971, Andrén and Lagerlöf 1983) are usually positively correlated with soil organic matter content. At both sites microbial C and nematode and acari populations were similar to values reported in the literature, however protozoa populations were higher and collembola populations were lower at Breton (Table II.6).

We did not measure bacterial and fungal biomass in these soils. Soil fungi typically have greater C/N ratios than bacteria (McGill et al. 1981, Hunt et al. 1987). Ingham and Horton (1987) found that the ratios of CO_2-C to inorganic N released during the 10-day postfumigation period of the chloroform fumigation technique correlated with prefumigation fungal to bacterial biomass ratios. We infer a greater fungal/bacterial ratio in the 0-10 cm depth at Ellerslie based on two arguments. Firstly, the ratio of the flush of CO_2-C/ flush of mineral N (NH_4-N + NO_3-N) over the 10-day incubation following fumigation (flush C/N ratio) (Figure II.3 and Table II.4) was greater at Ellerslie compared to Breton. Secondly, lower availability of water at Ellerslie (the soil water contents in the 0-10 cm interval corresponded to matric potentials ~-1.5 MPa on date 2 and date 3) than Breton during 1986 would favor fungal growth since most soil bacterial activity is limited at potentials ≤-1.5 MPa (Griffin 1981). Fungi tolerate potentials as low as -8 MPa (Bamforth 1985) and may have been physiologically more active in this soil. Water contents would have to drop to 11% to have the same effect at Breton. This did not occur during the growing season in 1986.

Table II.6. Comparison of Microbial C and Faunal Populations with Values Reported for Cultivated Soils

Depth (cm)	Mean	Site Description[a], Crop and Sampling Date(s)	Reference
		Microbial C (μg g^{-1} soil) by CFIM[b]	
0-7.5	260	Swift Current, Saskatchewan; L-SiL; 8 year cont wheat; Oct 1982	Biederbeck et al (1984)
0-7.5	469	Indian Head, Saskatchewan; C; wheat; June 1982	Campbell et al (1986)
0-15	310	Breton, Alberta; SiL; oats of 5 year cereal-forage rotation; July-Aug 84	Fyles et al (1988)
0-5	166	Breton, Alberta; SiL; barley of 5 year rotation; May-Oct 1982	McGill et al (1986)
0-20	276	Uppsala, Sweden; SCL; fertilized cereal; Nov 1982	Schnürer et al (1985)
0-10	242	Breton, Alberta; barley; 1986	This study
0-10	533	Ellerslie, Alberta; barley; 1986	This study
		Protozoa (no. g^{-1} soil) by MPN[c]	
0-20	4×10^4	Uppsala, Sweden; SCL; fertilized cereal; Nov 1982	Schnürer et al (1985)
0-10	15×10^4	Kjettslinge, Sweden; L; irrigated barley; July 1982, 2 day sampling intervals	Schnürer et al (1986a)
0-10	12×10^4	Kjettslinge, Sweden; L; barley; Sept 81	Schnürer et al (1986b)
0-10	5×10^4	Kjettslinge, Sweden; L; barley; Sept 83	Schnürer et al (1986b)
0-10	19×10^5	Breton, Alberta; barley; 1986	This study
0-10	13×10^5	Ellerslie, Alberta; barley; 1986	This study
		Nematodes (no. m^{-2})	
0-20	1.7×10^6	As site, Sweden; L; barley undersown with ley; Sept 1977	Andrén and Lagerlöf (1983)
0-20	3.1×10^6	Lanna site, Sweden; C; barley in barley-oat rotation; Oct 1977	Andrén and Lagerlöf (1983)
0-20	6.2×10^6	Kjettslinge, Sweden; L; barley; May-Dec 1982	Boström and Sohlenius (1986)
0-15	4.1×10^6	Breton, Alberta; SiL; oats of 5 year cereal-forage rotation; July-Aug 84	Fyles et al (1988)
0-20	7.3×10^6	Kjettslinge, Sweden; L; barley; May-Sept 1981	Sohlenius and Boström (1986)
0-20	1.0×10^6	Breton, Alberta; barley; 1986	This study
0-20	5.1×10^6	Ellerslie, Alberta; barley; 1986	This study

[a] C, clay; L, loam; S sandy; Si, silty

[b] CFIM=chloroform fumigation incubation method

[c] MPN=most probable number

Table II.6. Continued

Depth (cm)	Mean	Site Description[a], Crop and Sampling Date(s)	Reference
		Acari (no. m^{-2}) by High Gradient Heat Extraction	
0-10	4.6×10^4	As site, Sweden; L; barley undersown with ley; Sept 1977	Andrén and Lagerlöf (1983)
0-10	2.9×10^4	Lanna site, Sweden; C; barley in barley-oat rotation; Oct 1977	Andrén and Lagerlöf (1983)
0-5	4.1×10^4	Athens, Georgia; SCL; sorghum of sorghum, soybean, rye rotation; May-Dec 83	House and Parmelee (1985)
0-10	2.0×10^4	Breton, Alberta; barley; 1986	This study
0-10	4.3×10^4	Ellerslie, Alberta; barley; 1986	This study
		Collembola (no. m^{-2}) by High Gradient Heat Extraction	
0-10	1.1×10^4	As site, Sweden; L; barley undersown with ley; Sept 1977	Andrén and Lagerlöf (1983)
0-10	1.2×10^4	Lanna site, Sweden; C; barley in barley-oat rotation; Oct 1977	Andrén and Lagerlöf (1983)
0-5	6.2×10^3	Athens, Georgia; SCL; sorghum; May-Dec 83	House and Parmelee (1985)
0-10	0.9×10^3	Breton, Alberta; barley; 1986	This study
0-10	4.3×10^3	Ellerslie, Alberta; barley; 1986	This study
		Acari + Collembola (no. m^{-2}) by High Gradient Heat Extraction	
0-15	8.1×10^4	Breton, Alberta; SiL; oats of 5 year cereal-forage rotation; July-Aug 84	Fyles et al (1988)

[a] C, clay; L, loam; S sandy; Si, silty

The trends in microbial C and faunal populations are influenced by many factors such as water content, aeration, temperature, C supply, nutrient availability, grazing and predation. In the remainder of this section we propose a scenario of the events that lead to the population dynamics of soil fauna at Breton and Ellerslie. The hypothesis was developed by correlation and analysis of trends.

Excess water at Breton in July caused premature senescence of barley plants. Both shoot mass and root mass declined during the remainder of the season. Soil C, root exudation and death contributed to a readily available C pool which stimulated microbial activity. Water content and %WFP were high enough that bacterial activity was not limited. High bacterial biomass and relatively high %WFP allowed protozoan populations to increase in number throughout the sampling period from the potentially low levels that might have existed during the anaerobic conditions in July. The increase in nematode population throughout the study, even though primary production was decreasing, may be related to their slow lifecycle. Favorable abiotic conditions and a high abundance of bacteria, or an easy access to them, allowed bacterial feeding nematodes to reach greater proportions at Breton than at Ellerslie. The increase in acari populations in late September may have been due to a combination of ample food supply and favorable abiotic conditions after the cool wet conditions in July. The low counts in October of the present study may have been caused by death or migration due to the combination of cold, wet soil and reduced plant cover.

Lesser rainfall at Ellerslie compared to Breton and the greater porosity of the Black Chernozemic soil probably prevented development of anaerobic conditions. Shoot mass remained relatively constant from late July to late September. Root mass decreased in the surface depth when soil water potential ≤-1.5 MPa. Though soil water content was not significantly different at both sites, there was less available water at Ellerslie. Percent water-filled porosity was significantly lower in the Black Chernozemic soil and soil water potential was near -1.5 MPa in the 0-20 cm interval in August and early September. This probably restricted bacterial activity more than fungal activity. Water stress did not seem to severely limit total nematode populations or acari populations since both groups increased throughout the study. The positive correlation of collembola with soil water, and the decrease in numbers in early September followed by the large increase in late September, suggest that collembola were controlled by soil water content (Fjellberg 1985). It is likely that the dry conditions caused these animals to migrate deeper into the profile in early September and then later return to the surface when soil was more moist (Whitford et al.1981).

Relationship between Composition of Soil Biota and C Decomposition

C availability to microorganisms can be measured by quantifying flux from kinetically active pools. Pool sizes can be expressed on a soil basis or on soil C basis. The latter is a better estimate of availability of C to microorganisms (Dinwoodie and Juma 1988a). The metabolic activity of the microbial communities in the two soils were quite different. The organisms in the Gray Luvisol respired 1.8-fold more CO_2-C per unit microbial C. Using ^{14}C tracer Dinwoodie and Juma (1988b) estimated that 17% of the microbial C was active at Ellerslie compared to 43% at Breton. A greater proportion of bacteria at Breton would account for the greater CO_2-C evolution per unit microbial C since bacteria usually have greater growth and respiration rates than fungi (Griffin 1985). In addition, greater protozoan numbers (no. g^{-1} soil C) at Breton would exert a greater grazing pressure on bacteria at Breton compared to Ellerslie and contribute to a greater CO_2-C evolution per unit microbial C. At Ellerslie a much larger proportion of nematodes were plant parasites than at Breton (data not shown) such that a smaller proportion were dependent on soil and microbial C for growth and reproduction. Thus, organism composition at the two sites affected C decomposition and C loss from the soil.

Implications

The results of this study show that soil type and associated site conditions affected the detrital food webs at the two sites. Soil organic matter quality in various soils is different and this, along with different environmental conditions, dictates the relative activity of the web and its composition. More work is required to investigate the effects of soil properties on the size, structure and activity of detrital food webs. Such knowledge could lead to an increased understanding of the behavior of C and N in soils and be used to develop specific management practices for different soils.

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Chapter 3. Shoot, Root, Soil and Microbial Nitrogen Dynamics in Two Contrasting Soils Cropped to Barley*

Introduction

Soils belonging to the Chernozemic and Luvisolic Orders are important for cereal production in Alberta and represent two extremes of natural fertility; biological, physical and chemical properties; and crop productivity. Tracer studies have shown that up to 40-70% of fertilizer N is taken up by annual crops, 10-30% remains in the soil and the rest is unaccounted for (Stevenson 1986). Fate of fertilizer N is affected by many factors such as environmental conditions, cropping practices, placement and timing (Olson and Kurtz 1982). Although the recovery of fertilizer N in harvested crops has been measured for a variety of conditions in these soils, information on the dynamics of N uptake by cereals over the growing season is scarce. More information is needed for specific agroecosystems throughout Alberta in order to develop more widely applicable concepts.

A very small fraction of fertilizer N remains as NH_4-N and NO_3-N in cropped soils at the end of the season (Stevenson 1986). Because soluble inorganic N is subject to crop uptake, leaching, denitrification, volatilization, immobilization, and clay fixation, it is important to determine the occurrence of major processes involved in partitioning of mineral N over the growing season.

Mineralization-immobilization turnover (MIT) studies using classical fractionation, acid hydrolysis in various particle size fractions and in whole soil, and the kinetics of biologically meaningful pools have been reviewed by Juma and McGill (1986). MIT is affected by architecture and location of substrates (McGill and Myers 1987), soil management (Campbell and Souster 1982), environmental conditions (Schimel and Parton 1986), texture and mineralogy (Monreal et al. 1981, van Veen et al. 1985), available energy/ N ratio (Jansson 1958) and by interactions of the decomposer foodweb in soils (Coleman et al. 1984). There is a need to characterize the organic N into biologically meaningful fractions in order to predict MIT effectively. Soil organic N can be kinetically separated into an active N phase and a passive N phase (Jansson 1958). The active N phase is made up of living microbial N and non-microbial active N (NMAN), consisting of microbial metabolites and/or recently stabilized microbial residues (Paul and Juma 1981). The NMAN has a similar enrichment as microbial N in most isotope studies. Microbial N and NMAN are the major sources and sinks of mineral N (Juma and Paul 1984). Active N pools, estimated by isotope dilution techniques, account for 5-15% of soil N (Jansson 1958, Paul and Juma 1981).

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Knowledge of the size and activity of the active N pools in various soils is needed for predicting soil N availability for crops over the growing season, but is in itself insufficient. The flux of N is the product of N pool sizes and their specific mineralization rate constants. Consequently, a soil with a low organic N content, but a high specific mineralization rate, may mineralize as much N as a soil with a high organic N content and a low specific mineralization rate. Therefore comparison of pool sizes between soils does not provide enough information to understand N dynamics.

The objectives of this study were to determine whether: (i) N dynamics and distribution and recovery of urea-^{15}N in the barley-soil system of a Black Chernozemic site differs from that of a Gray Luvisolic site; and whether (ii) the proportion of total N in kinetically active pools and N flux through them differ in these soils.

Materials and Methods

Site Description and Soils: The sites and soil properties have been described in Chapter 2 and Table II.1.

Experimental Design and Sampling Schedule: The experiment was a factorial split-plot design with three blocks at two sites. Four open ended cylinders (20 cm internal diameter, 30 cm long) were pressed into each block in late May 1986. On May 28 1986 the cylinders were seeded (8 seeds per cylinder) to barley (*Hordeum vulgare* L., cv. Empress) and injected with 10 mL of urea solution (23 mg N mL^{-1}; 7.5 g N m^{-2}; 5.2% ^{15}N excess) at a depth of 2 cm with a syringe. The area surrounding the cylinders was also seeded to barley and fertilized with urea (7.7 g N m^{-2}) and triple superphosphate (4.7 g P m^{-2}) three days prior to the installation of the cylinders. No herbicides were used and the few weeds that appeared were included in the plant analyses.

At each sampling date the shoot material from a cylinder in each block was cut at ground level. Cylinders for each sampling date were excavated from each block and soil was divided into three depths: 0-10, 10-20 and 20-30 cm in the laboratory. Roots were separated from soil by hand. Soil samples were stored moist at 5°C for later determination of microbial biomass and mineral N. Subsamples were dried for total N determination. At Ellerslie plots were sampled on July 31, August 18, September 8 and September 29, which corresponded to 64, 82, 103 and 124 days after sowing, respectively. At Breton plots were sampled on August 11, September 1, September 22 and October 20, which corresponded to 75, 96, 117 and 145 days after sowing. Early season samples were not taken due to wet conditions and laboratory renovations. The last sampling date at Breton was also delayed because of wet field conditions.

Analyses: Shoot and root material was dried at 70°C and weighed. Shoots were ground to 20 mesh in a Wiley mill and root material was ground with a mortar and pestle. Total N was determined on soil, shoot and root (0-10, 10-20 cm) samples by semi-micro Kjeldahl analysis with steam distillation after reduction of NO_3-N with iron (Bremner and Mulvaney 1982).

Soil mineral N was determined on sieved soil (10 mesh) at the time of sampling. Soil was extracted in a 2M KCl mixture (25g soil: 125 mL solution) for one hour prior to filtering through Whatman no. 42 filter paper. The extract was frozen until the time of analysis. Technicon AutoAnalyzers were used to determine NH_4-N (Industrial Method No. 98-70W, 1973) and NO_3-N (Industrial Method No. 497-77A, 1977). Subsamples for ^{15}N analysis were treated with MgO, steam distilled, titrated, spiked with 0.5 M H_2SO_4 and dried. NO_3-N was determined by reduction to NH_4-N with Devarda's alloy prior to distillation.

Microbial N (B_n) was determined by the method of Jenkinson and Powlson (1976) on moist sieved (10 mesh) soil adjusted to 55% water holding capacity: $B_n = F_n/K_n$, where F_n is {(mineral N (NH_4-N + NO_3-N) in fumigated soil incubated for 10 days)-(mineral N in non-fumigated soil incubated for 10 days)} and Kn=0.68 (Shen et al. 1984). The change in mineral N during the incubation of the non-fumigated samples was used as a measure of net N mineralization.

For isotope ratio analysis of various N fractions, NH_4-N in titrated samples was oxidized to N_2 using LiOBr (Porter and O'Deen 1977) and isotope analysis was determined using a Micromass 602C double collector mass spectrometer. Microbial ^{15}N excess was assumed to be the same as the ^{15}N excess of NH_4-N extracted from fumigated samples after a 10-day incubation. Non-microbial organic ^{15}N was calculated by subtracting the sum of microbial ^{15}N and mineral ^{15}N from total soil ^{15}N. Non-microbial active N (NMAN) was calculated by the method of Paul and Juma (1981):

NMAN= Non-microbial organic ^{15}N/ Atom % excess of microbial N.

Statistical analyses: Analysis of variance was performed on all experimental variables using UANOVA, a multivariate analysis of covariance SPSSx program developed at the University of Alberta (Taerum, personal communication). The procedure gives probabilities which are adjusted for unequal variances of the means (Greenhouse and Geisser 1959).

Results

Shoot, Root and Soil N Trends

Shoot N (Table III.1) was on average 3.1-fold greater at Ellerslie than Breton and peaked at the third sampling date at Ellerslie and at the second sampling date at Breton. Average shoot N concentration was 1.9-fold greater at Ellerslie than Breton and decreased with time at Ellerslie but increased at Breton. Average shoot C/N ratios (Table III.1) were lower at Ellerslie (19.7) compared to Breton (31.7) and showed opposite trends over time at the two sites.

Root N decreased with depth but the decrease was more at Breton than at Ellerslie. Root N concentration (Table III.1) was not significantly different between sites, dates or depths. Root C/N ratios (Table III.1) were not significantly different between sites in the 0-10 cm interval but ratios peaked at the second sampling date at both sites.

Table III.1. Total N Budget and Barley N Concentration and C/N Ratio at Ellerslie and Breton (means of 3 replicates)

		Days After Sowing[a]			
Variable	Depth (cm)	64/75	82/96	103/117	124/145
		Ellerslie			
Shoot N (g m^{-2})		13.3	12.0	18.9	12.0
Shoot N (%) Concentration		2.50	2.08	2.14	1.79
Shoot C/N Ratio		14.9	20.5	19.7	23.7
Root N	0 - 10	1.1	0.5	0.7	0.9
(g m^{-2})	10 - 20	0.2	0.1	0.2	0.2
Root N (%)	0 - 10	1.04	1.30	1.56	1.47
Concentration	10 - 20	1.24	1.13	1.42	1.76
Root C/N Ratio	0 - 10	17.5	23.6	19.2	21.8
Soil N	0 - 10	462	450	462	448
(g m^{-2})	10 - 20	534	535	539	482
	20 - 30	462	477	515	502
Total Soil N		1458	1462	1516	1432
		Breton			
Shoot N (g m^{-2})		4.3	5.4	4.7	3.6
Shoot N (%) Concentraton		0.71	1.21	1.15	1.37
Shoot C/N Ratio[d]		37.6	30.3	33.5	25.2
Root N	0 - 10	0.8	0.9	0.9	0.8
(g m^{-2})	10 - 20	0.1	0.03	0.2	0.1
Root N (%)	0 - 10	1.24	1.28	1.24	1.56
Concentration	10 - 20	1.45	0.47	1.62	1.32
Root C/N Ratio	0 - 10	21.1	24.0	19.2	19.2
Soil N	0 - 10	195	228	203	197
(g m^{-2})	10 - 20	206	214	211	218
	20 - 30	140	206	149	146
Total Soil N		541	648	563	561

In this and subsequent tables:

[a] Sampling date for Ellerslie and Breton respectively

Table III.1. Continued

Summary of ANOVA[a]

Source of Variation	Shoot N	Shoot N (%)	Shoot C/N	Root N	Root N (%)	Root C/N	Soil N
Site (S)	*	***	**	ns	ns	ns	***
Date (D)	*	ns	ns	ns	ns	*	ns
S x D	*	**	*	ns	ns	ns	ns
Depth (Dp)				***	ns		ns
S x Dp				*	ns		ns
D x Dp				ns	ns		ns
S x D x Dp				ns	ns		ns

In this and subsequent tables:

[a] The difference between means is significant at: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$

Total soil N (Table III.1) was 2.5-fold greater at Ellerslie compared to Breton. The N concentration decreased with depth (Table II.1) but total soil N (g m^{-2}) did not decrease with depth because bulk density increased with depth (Table II.1).

Mineral N Trends

NH_4-N decreased with time and increased with depth at both sites (Table III.2). NO_3-N and total mineral N were greatest in the 20-30 cm interval at Ellerslie but was similar for all depths at Breton. NO_3-N decreased on date two, increased on date three and decreased on the last sampling date at both sites.

Microbial N Trends and Net N Mineralization in the Laboratory

Microbial N (Table III.3) was on average 1.4-fold greater at Ellerslie compared to Breton. Microbial N declined over the first three dates at Ellerslie and increased on the last date; in contrast, it peaked at Breton at date 2. Microbial N decreased with depth at both sites.

Table III.2. Mineral N (g m^{-2}) at Ellerslie and Breton (means of 3 replicates)

N Pool	Depth (cm)	Days After Sowing 64/75	 82/96	 103/117	 124/145
		Ellerslie			
NH_4-N	0 - 10	0.34	0.33	0.44	0.16
	10 - 20	0.40	0.32	0.27	0.18
	20 - 30	0.67	0.63	0.70	0.54
NO_3-N	0 - 10	0.79	0.46	0.87	0.52
	10 - 20	0.80	0.52	0.62	0.72
	20 - 30	2.87	1.49	1.55	1.02
Mineral N	0 - 10	1.12	0.79	1.31	0.68
	10 - 20	1.20	0.84	0.90	0.89
	20 - 30	3.54	2.12	2.25	1.56
		Breton			
NH_4-N	0 - 10	0.29	0.35	0.17	0.15
	10 - 20	0.49	0.33	0.22	0.33
	20 - 30	0.76	0.55	0.39	0.40
NO_3-N	0 - 10	0.90	0.54	0.52	0.43
	10 - 20	0.72	0.48	0.69	0.41
	20 - 30	0.49	0.48	0.53	0.33
Mineral N	0 - 10	1.19	0.89	0.68	0.57
	10 - 20	1.21	0.81	0.91	0.74
	20 - 30	1.24	1.03	0.93	0.73

Summary of ANOVA

Source of Variation	NH_4-N	NO_3-N	Mineral N
Site (S)	ns	ns	ns
Date (D)	*	*	*
S x D	ns	ns	ns
Depth (Dp)	***	ns	*
S x Dp	ns	ns	ns
D x Dp	ns	ns	ns
S x D x Dp	ns	ns	ns

Table III.3. Microbial N and Net N Mineralized (g m^{-2}) from Non-Fumigated Soil Samples during 10-day Incubation (means of 3 replicates)

N Pool	Depth (cm)	Days After Sowing 64/75	82/96	103/117	124/145
		Ellerslie			
Microbial N	0 - 10	6.52	4.49	5.10	5.72
	10 - 20	5.75	5.37	3.73	5.08
	20 - 30	3.99	2.29	2.01	3.02
Net N Mineralized	0 - 10	0.87	0.92	1.09	0.82
	10 - 20	0.64	0.66	0.67	0.37
	20 - 30	0.70	0.47	0.40	0.63
		Breton			
Microbial N	0 - 10	3.49	5.47	3.41	4.23
	10 - 20	2.98	4.57	2.83	3.27
	20 - 30	1.80	3.24	1.36	1.73
Net N Mineralized	0 - 10	0.78	0.88	0.95	0.66
	10 - 20	0.42	0.51	0.47	0.36
	20 - 30	0.12	0.39	0.14	0.17

Summary of ANOVA

Source of Variation	Microbial N	Net N Mineralized
S (S)	*	ns
Date (D)	ns	ns
S x D	*	ns
Depth (Dp)	***	***
S x Dp	ns	ns
D x Dp	ns	ns
S x D x Dp	ns	ns

Net N mineralization was 1.4-fold greater in the soil from Ellerslie when expressed per gram soil (data not shown), however it was not significantly different between the two soils when expressed on an area basis (g m^{-2}) (Table III.3). Net mineralization (g m^{-2}) generally declined with depth at both sites.

Table III.4. Percent ^{15}N Recovery in the Plant-Soil System at Ellerslie and Breton (means of 3 replicates)

N Pool	Depth (cm)	Days After Sowing			
		64/75	82/96	103/117	124/145
			Ellerslie		
Shoot N		41.4	33.9	40.7	26.4
Root N	0 - 10	1.8	1.2	1.6	1.5
	10 - 20	0.3	0.2	0.3	0.3
Total Soil N	0 - 10	19.0	22.2	20.6	16.4
	10 - 20	3.4	6.1	5.1	4.4
	20 - 30	2.4	1.1	1.4	1.2
		24.8	29.4	27.1	22.0
Total Recovery		68.3	64.7	69.7	50.2
			Breton		
Shoot N		20.5	19.7	17.9	11.3
Root N	0 - 10	3.5	3.3	3.1	2.4
	10 - 20	0.5	0.1	0.4	0.2
Total Soil N	0 - 10	21.7	30.2	32.3	22.6
	10 - 20	7.0	7.7	6.3	3.6
	20 - 30	3.4	3.5	1.9	1.3
		32.1	41.4	40.5	27.5
Total Recovery		56.6	64.5	61.9	41.4

Summary of ANOVA

Source of Variation	Shoot ^{15}N Recovery	Root ^{15}N Recovery	Soil ^{15}N Recovery
Site (S)	*	**	**
Date (D)	*	ns	ns
S x D	ns	ns	ns
Depth (Dp)		***	***
S x Dp		***	*
D x Dp		ns	ns
S x D x Dp		ns	ns

^{15}N Recovery in the Plant-Soil System

Shoot ^{15}N was the only ^{15}N pool measured which significantly changed over the sampling dates (Table III.4). Shoot ^{15}N recoveries were greater at Ellerslie (36%) compared to Breton (17%). At both sites shoot ^{15}N recoveries declined such that recoveries at date four were much lower than those at date one. Shoot ^{15}N was the largest pool at Ellerslie however total soil ^{15}N was the largest pool at Breton. On average, root ^{15}N was 1.9-fold greater and soil ^{15}N was 1.4-fold greater at Breton compared to Ellerslie. Root ^{15}N decreased more with depth at Breton than at Ellerslie. Soil ^{15}N decreased with depth at both sites but declined more at Breton. On average, total recoveries of applied urea ^{15}N in the soil-plant system were greater at Ellerslie (63%) than at Breton (56%).

Distribution of ^{15}N in Soil Fractions

^{15}N recovery as mineral N (Table III.5) was 1.6- to 2.6-fold greater at Ellerslie than at Breton. There was no difference in ^{15}N recovery in the microbial biomass between sites. Microbial ^{15}N decreased with depth at both sites but declined more at Ellerslie.

The largest pool of ^{15}N in the upper two depths was non-microbial organic N (Table III.5). This pool was greater at Breton (31%) than Ellerslie (20%). Non-microbial organic ^{15}N recovery decreased with depth at both sites but the decline was less with depth at Ellerslie.

Microbial and Mineral N as a Proportion of Soil N

In contrast to microbial N size (g m^{-2}, Table III.2), microbial N expressed as a proportion of soil N (Table III.6), was on average 1.7-fold greater at Breton than at Ellerslie. At Ellerslie microbial N over all depths accounted for 0.9% of total soil N but contained 11.8% of applied ^{15}N.

Table III.5. Percent ^{15}N Recovery in Soil N Pools at Ellerslie and Breton (means of 3 replicates)

N Pool	Depth (cm)	Days After Sowing			
		64/75	82/96	103/117	124/145
		Ellerslie			
Total Mineral N	0 - 10	1.28	1.28	1.60	0.40
	10 - 20	1.67	0.70	0.58	0.57
	20 - 30	2.04	1.40	0.93	0.80
Microbial N	0 - 10	3.14	1.82	2.29	2.08
	10 - 20	0.55	0.45	0.29	0.30
	20 - 30	0.26	0.23	0.16	0.22
Non-Microbial Organic N	0 - 10	14.56	19.08	16.70	13.95
	10 - 20	1.22	4.95	4.27	3.53
	20 - 30	0.12	0.00	0.31	0.18
		Breton			
Total Mineral N	0 - 10	1.24	0.72	1.02	0.30
	10 - 20	0.69	0.40	0.58	0.17
	20 - 30	0.67	0.19	0.33	0.24
Microbial N	0 - 10	2.15	1.77	1.66	1.36
	10 - 20	1.24	0.97	0.92	0.44
	20 - 30	0.60	0.35	0.24	0.23
Non-Microbial Organic N	0 - 10	18.65	27.72	29.65	20.97
	10 - 20	4.89	6.31	4.62	3.00
	20 - 30	2.05	2.93	1.47	0.86

Summary of ANOVA

Source of Variation	Total Mineral ^{15}N Recovery	Microbial ^{15}N Recovery	Non-Microbial Organic ^{15}N Recovery
Site (S)	*	ns	**
Date (D)	ns	ns	ns
S x D	ns	ns	ns
Depth (Dp)	ns	***	***
S x Dp	ns	*	**
D x Dp	ns	ns	ns
S x D x Dp	ns	ns	ns

Table III.6. Microbial N and Mineral N Expressed as a Proportion of Soil N; and, Microbial ^{15}N and Mineral ^{15}N Expressed as a Proportion of Soil ^{15}N at Ellerslie and Breton (means of 3 replicates)

N Pool	Depth (cm)	Days After Sowing			
		64/75	82/96	103/117	124/145
		Ellerslie			
		% of soil N			
Microbial N	0 - 10	1.40	1.00	1.11	1.28
	10 - 20	1.07	1.12	0.70	1.08
	20 - 30	0.86	0.48	0.40	0.55
Mineral N	0 - 10	0.25	0.18	0.28	0.15
	10 - 20	0.23	0.16	0.17	0.19
	20 - 30	0.77	0.42	0.43	0.31
		% of soil ^{15}N			
Microbial ^{15}N	0 - 10	16.6	8.2	11.5	13.0
	10 - 20	16.2	11.8	7.6	7.9
	20 - 30	14.1	3.8	14.1	17.0
Mineral ^{15}N	0 - 10	6.9	6.0	7.9	2.6
	10 - 20	45.8	21.2	12.9	15.7
	20 - 30	70.9	22.4	64.3	71.3
		Breton			
		% of soil N			
Microbial N	0 - 10	1.78	2.39	1.68	2.14
	10 - 20	1.57	2.13	1.34	1.51
	20 - 30	1.14	1.51	0.88	1.13
Mineral N	0 - 10	0.60	0.39	0.34	0.29
	10 - 20	0.58	0.37	0.43	0.34
	20 - 30	0.94	0.58	0.66	0.59
		% of soil ^{15}N			
Microbial ^{15}N	0 - 10	10.3	6.0	5.0	5.9
	10 - 20	17.8	10.8	13.6	12.8
	20 - 30	16.9	8.5	12.7	12.3
Mineral ^{15}N	0 - 10	4.5	2.4	3.1	1.3
	10 - 20	10.1	5.6	8.7	4.7
	20 - 30	21.3	6.2	17.3	16.7

Table III.6. Continued

	Summary of ANOVA			
Source of Variation	Microbial N / Soil N	Microbial N-15 / Soil N-15	Mineral N / Soil N	Mineral N-15 / Soil N-15
Site (S)	**	ns	**	*
Date (D)	**	ns	**	ns
S x D	**	ns	ns	ns
Depth (Dp)	***	ns	**	*
S x Dp	ns	ns	ns	ns
D x Dp	ns	ns	ns	ns
S x D x Dp	ns	ns	ns	ns

At Breton microbial N accounted for 1.6% of total soil N and contained 11.1% of applied ^{15}N. Microbial N expressed as a proportion of soil N (%) changed with date and decreased with depth. Microbial ^{15}N, expressed as a proportion of soil ^{15}N, did not significantly differ with respect to site, date or depth.

Mineral N was greater at Breton than Ellerslie when expressed as a proportion of soil N (Table III.6). Mineral N (% of soil N) varied with date and increased with depth. A large proportion of soil ^{15}N was present as mineral N at both sites despite the small size of the mineral N pool (Table III.6). A larger proportion of soil ^{15}N was present as mineral N at Ellerslie than at Breton. The proportion of mineral ^{15}N to soil ^{15}N increased with depth, especially at Ellerslie where mineral ^{15}N accounted for about 57% of soil ^{15}N in the 20-30 cm interval.

Absolute versus Proportional N Pool Sizes and Net N Mineralization in 0-10 cm Soil Samples

Soil N (g m^{-2}) and net N mineralization (µg g^{-1} soil 10 days^{-1}) were significantly greater in soil samples from Ellerslie than Breton in the 0-10 cm interval (Table III.7). The only variable which showed a significant site by date interaction in Table III.7 was microbial N (% of soil N) ($p \leq 0.007$). Microbial N (g m^{-2}),

Table III.7. Absolute versus Proportional N Pool Sizes and Net N Mineralized in 0-10 cm Soil from Ellerslie and Breton (means of 12 replicates)

Variable	Site: Ellerslie	Site: Breton	Significance of Site Effect
Absolute (per m^2 or g soil basis)			
Soil N (g m^{-2})	456	206	***
Microbial N (g m^{-2})	5.5	4.1	ns
NMAN[a] (g m^{-2})	46.1	66.2	ns
Total mineral N (g m^{-2})	0.98	0.83	ns
Net N mineralized (μg g^{-1} soil 10 $days^{-1}$)	10.4	7.2	*
Net ^{15}N mineralized (μg 100 g^{-1} soil 10 $days^{-1}$)	4.2	3.0	ns
Proportional (per unit N basis)			
Microbial N (% of soil N)	1.2	2.0	**
NMAN[a] (% of soil N)	10	32	**
Total mineral N (% of soil N)	0.21	0.40	**
Net N mineralized (mg g^{-1} microbial N 10 $days^{-1}$)	17	20	ns
Net N mineralized (mg g^{-1} NMAN 10 $days^{-1}$)	2.4	1.5	*
Net N mineralized (mg g^{-1} soil N 10 $days^{-1}$)	0.20	0.39	**
Net ^{15}N mineralized (% of soil ^{15}N 10 $days^{-1}$)	4.8	3.2	ns

[a] Non-microbial active N

NMAN (g m^{-2}), total mineral N (g m^{-2}) and net ^{15}N mineralization (μg g^{-1} soil 10 $days^{-1}$) were not significantly different between the two soils.

Microbial N, NMAN and mineral N made up a larger proportion of soil N at Breton compared to Ellerslie. Net N mineralization was not different between soils when expressed as a proportion of microbial N, however it was greater in soil from Ellerslie when expressed as a proportion of NMAN. Net N mineralization was greater in soil from Breton when expressed as a proportion of total soil N. Net ^{15}N mineralization did not significantly differ between soils when expressed as a proportion of soil ^{15}N.

Discussion

N Dynamics in the Two Agroecosystems

Differences in environmental conditions and soil properties at the two sites resulted in different N dynamics and different distribution and recovery of urea-^{15}N. Rainfall was probably the major environmental factor because Breton received approximately 285 mm in July, almost three times the 30-year mean of 95 mm while Ellerslie received 126 mm, approximately 1.5 times the 30-year mean of 85 mm. Wet conditions affected the plant growth at Breton, causing premature senescence. In July the plants at Breton were pale green with a shoot N concentration of 0.71%, which is considered to be deficient (Olson and Kurtz 1982, Engel and Zubriski 1982). Shoot C/root C ratios at Breton were on average 60% of those at Ellerslie (Dinwoodie and Juma 1988) and may have been due to more favorable N status at Ellerslie. Low shoot ^{15}N recoveries at Breton compared to Ellerslie were due to less early season fertilizer uptake or greater loss of shoot ^{15}N through senescence (Sallam and Scott 1987). Higher root ^{15}N at Breton was probably due to reduced N translocation to the shoots due to wet conditions in July. Shoot N concentration declined as the plants matured at Ellerslie however increased at Breton as plants recovered from the wet conditions and possibly conserved N during premature senescence (Trought and Drew 1980). The large decline of shoot ^{15}N on date four at both sites indicates significant loss of N in the fall.

Availability of N to cereals is affected by dynamics within the mineral pools. Leaching losses occurred at both sites. Soil samples taken from below the cylinders (>30 cm) on date one (data not shown) at both sites had mineral ^{15}N abundances in excess of natural abundance. The decline of NO_3-N with depth at Breton was probably due to early season deep leaching accompanied by denitrification. Percent water-filled porosity was very high (>80%) all season in the 20-30 cm interval (Chapter 2) and the NO_3-N would have been prone to denitrification (Linn and Doran 1984) if C was available. Monreal and McGill (1983) found that denitrification at Breton may be restricted by low supply of water soluble organic C (WSOC). Dinwoodie and Juma (1988) found low WSOC at Breton, however small pool size does not necessarily imply low supply.

The greater recovery of mineral ^{15}N at Ellerslie despite a non-significant site effect in mineral N indicates that mineralization of immobilized ^{15}N was less or perhaps leaching and denitrification losses were greater at Breton. Similar trends between mineral ^{15}N and NO_3-N distribution indicate that most mineral ^{15}N was in the form of NO_3-N. The decline of mineral ^{15}N between date three and date four at both sites was probably due to leaching and/or denitrification since rainfall occurred during this time.

Despite greater absolute mineral N (g m^{-2}) at Ellerslie, a larger proportion of soil N existed as mineral N at Breton. Thus net N mineralization was proportionally greater, or losses and plant uptake of mineral N were proportionally less at Breton. At both sites the recovery of soil ^{15}N in the mineral pool increased with depth as mineral N was leached into the profile. The much greater proportion of soil ^{15}N as mineral N in the 20-30 cm interval at Ellerslie compared to Breton was probably due to greater losses at Breton.

Availability of mineral N is affected by mineralization and immobilization reactions mediated by the microbial biomass. Microbial N (g m^{-2}) was greater at Ellerslie because the soil had a much greater C and N content. This was expected since microbial biomass has been shown to correlate positively with total organic C and N in soil (Schnürer et al. 1985, Carter 1986). Microbial ^{15}N did not differ between sites even though microbial N was greater at Ellerslie indicating a greater

^{15}N enrichment at Breton. The decline over time in microbial ^{15}N at both sites, though not statistically significant, was due to dilution of the microbial N pool with non-labelled N as the microbial biomass turned over. The large recovery of ^{15}N in the microbial pool at both sites (4 to 18% of soil ^{15}N) indicates the importance of the microbial biomass being a significant sink and source of mineral N.

The large recovery of ^{15}N in the non-microbial organic N pool at Breton may be due to inherent differences in soil properties between the sites. Bergström (1986) found that mineral N supply decreased to pre-fertilization levels within three weeks of initial barley uptake. In the present study urea hydrolysis would have been essentially complete after three to four weeks and most fertilizer ^{15}N should have been partitioned between mineral N, microbial N and barley N before the heavy rains began in July. Thus the large recovery of ^{15}N in the non-microbial organic pool was due to greater early season immobilization at Breton. In addition mineralization is much slower under anaerobic conditions (Patrick Jr. 1982) and wet conditions at Breton may have slowed the conversion of organic ^{15}N into mineral forms. However, anaerobic conditions in the 0-10 cm interval at Breton only existed for a short time and it is doubtful that the legacy of these conditions existed until late summer. Perhaps early season wet conditions resulted in an increase in available C through root death, increased root exudation (Dinwoodie and Juma 1988), microbial fermentation or microbial death. Upon drying, re-immobilization of mineral ^{15}N into the soil microbial biomass and the non-microbial organic pool would occur. This is consistent with large soil ^{15}N recovery and low shoot ^{15}N recovery at Breton. Recoveries of ^{15}N in the non-microbial organic pool at Breton in 1984 under oats and alfalfa were similar to 1986 despite drier field conditions and addition of fertilizer ^{15}N when oat plants were one week old and alfalfa was in its second year (Juma, personal communication). Thus immobilization of fertilizer N occurs rapidly in the first four weeks after application.

Relationships Between Nitrogen, Carbon, Microbial and Faunal Activities

The two soils showed differences in the relative size of the kinetically active N pools and in relative mineralization rates. The microbial biomass and non-microbial active N (NMAN) pools are major sources and sinks of mineral N in soil. Though total N was much lower at Breton compared to Ellerslie, 34% of soil N in 0-10 cm samples was in the microbial and NMAN pools at Breton compared to 12% at Ellerslie. The results of Campbell and Souster (1982) are comparable to those of the present study. They found that potentially mineralizable N accounted for a larger proportion of soil N in cultivated Gray Luvisols compared to Black Chernozems.

Turnover rates were not determined for the two pools however there was no significant difference in net N mineralization between sites when expressed as a proportion of microbial N. Net N mineralization was less at Breton when expressed as a proportion of NMAN. However, the proportionally large size of the active pools at Breton and greater activity of the microbial biomass resulted in greater net N mineralization expressed as a proportion of total N at Breton compared to Ellerslie. This may have contributed to the greater proportion of soil N existing as mineral N at Breton during the summer of 1986.

Greater proportional C activity at Breton may be related to a relatively more active food web at Breton compared to Ellerslie (Chapter 2). A greater proportion of soil C was respired in the Gray Luvisol compared to the Black Chernozem in a 10-day laboratory incubation. Microbial C and most faunal counts were greater at Ellerslie when expressed per square meter; microbial C and protozoa were greater at

Breton and nematodes and microarthropods were not significantly different when expressed relative to soil C. Thus the C at Breton was proportionally more available to support biological activity than at Ellerslie. A hypothesis was proposed in Chapter 2 that the food web was more active at Breton compared to Ellerslie in 1986. Differences in detrital trophic web structure affect C and N turnover in the soil and the size and activity of the active N pools. Expressed proportionally, high C activity and a large decomposer food web (Chapter 2) kept a larger proportion of soil N in the soil organisms at Breton compared to Ellerslie (Table III.7). Rapid C turnover and greater microbial and faunal activity resulted in a greater proportional net N mineralization rate at Breton.

The greater activity of C and N at Breton may have been due to differences in the quality of organic matter, mineralogy, texture and organism activity at the two sites. A large proportion of the organic matter at Ellerslie may be stabilized and unavailable for rapid microbial decomposition. Pawluk (1986) hypothesized that the soil organic matter was resilient at Ellerslie because the soil had a high smectite content in the A horizon. This stabilizing influence may not have been as strong in the Ap at Breton due to its lower clay and smectite content (Pawluk 1980). The decomposer population is protected in fine textured soils (van Veen et al. 1984). Fine texture is associated with reduced mineralization of C and N from plant residues (Ladd et al. 1981, Ladd et al. 1985), and the turnover of C and N through the microbial biomass (Merckx et al. 1985, van Veen et al. 1985) and the proportion of soil N which is mineralizable (Campbell and Souster 1982).

Implications

This study showed that N dynamics in the Chernozemic and Luvisolic soils were markedly different. Since N dynamics are affected by physical, chemical and biological properties and associated processes, innovative management techniques are needed for conserving C and N in soils and for increasing N uptake by crops. This study also showed that comparison of N pool sizes only yields partial information on N dynamics. The comparison of flux between different N pools is important and should be considered when comparing nutrient dynamics in various soils and in evaluating different management practices.

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Chapter 4. Microbial Effects on Fertilizer N Recovery and Distribution in a Barley-Soil System

Introduction

Jansson (1958) used short-term ^{15}N studies to divide soil organic N into an active and a passive N phase. Paul and Juma (1981), using the chloroform fumigation incubation technique, extended Jansson's approach and divided the active N phase into a living component, microbial N, and a non-living component, non-microbial active N. Non-microbial active N consists of microbial metabolic products and recently stabilized materials. These microbial N and non-microbial active N pools are an important source of mineralizable N and accounted for more than 67% of the net N mineralized in a 12-week laboratory study (Paul and Juma 1981); and, they build rapidly when the ratio of available energy to N in the soil is high (Jansson 1958).

Microbial N serves two major purposes: (i) it is a source and sink of N, and (ii) it is a transformation agent for soil N (Anderson and Domsch 1980, Paul and Voroney 1980). Since microbes have a potentially high growth rate, they can assimilate available N rapidly and convert a major portion of this material into non-microbial organic N when sufficient C is available. The recovery of fertilizer N in microbial biomass is 1 to 3% after one field season but the recovery of fertilizer N in non-microbial organic N ranges from 14 to 21% (Chapter 3). The ratio of ^{15}N recovered in microbial biomass to ^{15}N recovered in non-microbial organic N represents the amount of N remaining in the microbial community relative to the total amount of N transformed by the community, assuming abiotic processes are negligible.

Mineralization and immobilization of N are continuous simultaneous processes that are mediated by soil organisms (Jansson and Persson 1982). Although microbial activity during the growing season results in net N mineralization, it may or may not coincide with crop uptake pattern (McGill and Myers 1987). Crop uptake is very low from emergence to fifth leaf stage but increases greatly from the latter stage to the heading stage. Fertilizer N is often subject to losses such as volatilization, leaching or denitrification before the period of greatest crop demand. One way to conserve fertilizer N in the plant-soil system is to immobilize it at the time of application by adding a readily available C source and to rely on the microorganisms to mineralize it concurrently with crop N demand during the season. Glucose may be an ideal C source because it results in a rapid immobilization of soluble N into microbial biomass and microbial residues. Rapidly cycling N pools may mineralize enough N to meet crop demand. Other substrates such as straw or cellulose (Azam et al. 1985, Azam et al. 1988, Bjarnason 1987) immobilize N over a prolonged period or result in formation of phenolic compounds in residues forming stable N products by condensation reactions (Haider et al. 1977, Kelly and Stevenson 1987), thereby making it difficult to evaluate the effect of substrate addition on net N mineralization and subsequent N cycling.

It is generally known that C addition causes net N immobilization in soil when the C/N ratio of the amendment exceeds 20:1 (Stevenson 1986); however, few studies report the effects glucose plus fertilizer N addition on plant growth and N partitioning (Schnürer and Rosswall 1987), and on N recovery within field soil (Kowalenko 1981). The effects of glucose addition on the partitioning of fertilizer N amongst plants, soil mineral N and active soil N pools in the field may provide an insight into N cycling and may aid in improving fertilizer use efficiency and in reducing losses of N to the environment.

The objectives of this field study were to evaluate the effects of glucose amendment, at the time of fertilization and planting, on the distribution of fertilizer ^{15}N at harvest amongst barley shoots and roots, soil mineral N, microbial N and non-microbial organic N. Soil sampled at the time of harvest was incubated in the laboratory to evaluate the long-term effects of glucose addition on microbial activity.

Materials and Methods

Site Description and Soils: The field experiment was conducted at the Ellerslie Research Station, near Edmonton in central Alberta. The site and soil properties have been described in detail in Chapter 2 and Table II.1.

Experimental Design and Sampling Schedule: Two open-ended cylinders (20 cm internal diameter and 30 cm long) were installed into each of three blocks in May 1986. On 28 May 1986 the cylinders were seeded to barley (*Hordeum vulgare* L. 'Empress') and fertilized with 10 mL of urea solution (23 mg N mL^{-1}; 7.5 g N m^{-2}, 5.2% ^{15}N excess) at a depth of 2 cm with a syringe. A 50 mL solution containing 4.9 g glucose-C (150 g C m^{-2}) was immediately poured on the soil surface of one cylinder in each block at both sites. One hundred mL of tap water were added to rinse the glucose solution into the soil. One hundred and fifty mL of tap water were added to the soil surface of each non-glucose replicate to evenly distribute urea throughout the upper soil layers. Assuming that all glucose-C remained in the surface 15 cm, the glucose amendment rate was 1 mg C g^{-1} soil. The area surrounding the cylinders was also seeded to barley and fertilized with urea (7.7 g N m^{-2}) and triple super phosphate (4.7 g P m^{-2}) two days prior to the installation of the cylinders. Herbicides were not used and the few weeds that appeared were included in the plant analyses.

Cylinders were sampled on 8 September 1986. At sampling, the shoot material was cut at ground level. Seed heads were still intact at harvest. Cylinders were excavated from each block and transported to the laboratory where the soil was divided into three depths: 0-10, 10-20 and 20-30 cm. Roots were separated from soil samples by hand and soil was stored at 5° C in plastic bags.

Shoot, Root, and Soil N Analyses: Shoot and root material was dried at 70°C and weighed. Shoots were ground to pass through a 1-mm sieve in a Wiley mill (A. H. Thomas Co., Philadelphia, PA) and root material was ground with a mortar and pestle. Total N was determined on soil, shoot and root (0-10, 10-20 cm) samples by semi-micro Kjeldahl analysis with steam distillation after reduction of NO_3-N with iron (Bremner and Mulvaney 1982).

Soil mineral N was determined on sieved (2 mm) soil at the time of sampling. Soil was extracted and analysed for NH_4-N and NO_3-N, as described in Chapter 3. Subsamples for ^{15}N analysis were treated with MgO, steam distilled, titrated, spiked with 0.5 M H_2SO_4 and dried.

For isotope ratio analysis of various N fractions, NH_4-N in titrated samples was oxidized to N_2 using LiOBr (Porter and O'Deen 1977) and isotope analysis was determined using a Micromass 602C double collection mass spectrometer (V. G. Isogas, Cheshire, England). ^{15}N excess of microbial N (see below) was assumed to be the same as the ^{15}N excess of NH_4 extracted from fumigated samples after a 10 day incubation. Non-microbial organic ^{15}N was calculated by subtracting the sum of microbial ^{15}N and mineral ^{15}N from total soil ^{15}N. It was assumed that the non-microbial organic ^{15}N remaining in soil at the end of a season was mainly present in the active N phase (Paul and Juma 1981).

Determination of Microbial Size, and Soil Activity under Laboratory Conditions: Microbial C and N measurements were performed in duplicate on sieved (2-mm) soil adjusted to 55% water holding capacity. Microbial respiration and microbial C and N were determined by the chloroform fumigation method (Jenkinson and Powlson 1976), as described in Chapter 2 and 3. The CO_2-C evolved from non-fumigated samples was used as a measure of soil respiration. The change in mineral N during the incubation of the non-fumigated samples was used as a measure of net N mineralization.

Statistics: Analysis of variance was performed on experimental variables using the SAS package GLM.

Results

Plant Growth

Shoot mass was 1.8-fold greater in the non-glucose treatment (NGT) compared to the glucose treatment (GT) (Table IV.1). Shoot N concentration (mean 1.8%) and total shoot N did not significantly differ between treatments, due to high variability of the results. Root mass was 1.9-fold greater in NGT than GT and declined more gradually with depth in the GT. Root N concentration (mean 1.5%) was not significantly different between treatments in the 0-20 cm interval, however, total root N was two-fold greater in NGT compared to GT. Shoot/root ratios were the same in both treatments.

Table IV.1. Dry Matter Yield and Barley N for Glucose Treatment (GT) and Non-Glucose Treatment (NGT) (means of 3 replicates)

Variable	Depth (cm)	GT	NGT
Shoot Mass (g m^{-2})		358	650
Shoot N (g m^{-2})	–	6.1	12
Root Mass (g m^{-2})	0 - 10	29	62
	10 - 20	9	12
	20 - 30	6	8
		44	82
Root N (g m^{-2})	0 - 10	0.4	0.9
	10 - 20	0.1	0.2
		0.5	1.1
Shoot Mass to Root Mass		8.1	8.1

Summary of ANOVA[a]

Source of Variation	Shoot Mass	Shoot N	Root Mass	Root N	Shoot Mass/Root Mass
Treatment (T)	*	ns	†	†	ns
Depth (Dp)			***	**	
T x Dp			**	†	

In this and all subsequent tables:

[a] The difference between means is significant at: †, $p \leq 0.10$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ns, not significant

Microbial C and N, and Mineral N

Microbial C, microbial N and total mineral N were not significantly different between treatments (Table IV.2). Microbial N was greatest in the 0-10 cm interval and declined with depth. Total mineral N (NH_4-N + NO_3-N) concentration increased with depth in both treatments.

^{15}N Recovery

Shoot ^{15}N recovery was 3.0-fold greater in NGT than GT (Table IV.3).

Table IV.2. Microbial C and N, and Mineral N (g m^{-2}) in Glucose Treatment (GT) Soil and Non-Glucose Treatment (NGT) Soil (means of 3 replicates)

Variable	Depth (cm)	GT	NGT
Microbial C	0 - 10	55.0	44.0
	10 - 20	48.1	47.4
	20 - 30	34.0	38.2
		137.1	129.6
Microbial N	0 - 10	6.8	5.7
	10 - 20	5.1	5.1
	20 - 30	2.7	3.0
		14.6	13.8
Mineral N	0 - 10	0.9	0.7
	10 - 20	1.0	0.9
	20 - 30	1.5	1.6
		3.4	3.2

Summary of ANOVA

Source of Variation	Microbial C	Microbial N	Mineral N
Treatment (T)	ns	ns	ns
Depth (Dp)	ns	**	***
T x Dp	ns	ns	ns

Approximately 10% of the shoot N was derived from the urea fertilizer in GT compared to 16% in NGT. ^{15}N recovery in roots was greater in NGT compared to GT, and was greater in the 0-10 cm interval compared to the 10-20 cm interval. Root ^{15}N declined more with depth in NGT compared to GT.

GT soil had higher ^{15}N recoveries than NGT soil for mineral N, microbial N and non-microbial organic N (Table IV.3). Microbial ^{15}N and non-microbial organic ^{15}N declined with depth more in GT soil compared to NGT soil. In both treatments, most soil ^{15}N recovery was in non-microbial organic N. ^{15}N recovered in GT soil was 3.3-fold greater than in NGT soil. Total soil ^{15}N declined with depth in both treatments, yet the decline was greater with depth in GT soil compared to NGT soil.

Table IV.3. Percent ^{15}N Recovery in Plant-Soil System for Glucose Treatment (GT) and Non-Glucose Treatment (NGT) (means of 3 replicates)

Variable	Depth (cm)	GT	NGT
Shoot N		8.8	26.4
Root N	0 - 10	0.7	1.5
	10 - 20	0.1	0.3
		0.8	1.8
Mineral N	0 - 10	1.0	0.4
	10 - 20	0.8	0.6
	20 - 30	0.5	0.8
		2.3	1.8
Microbial N	0 - 10	9.0	2.1
	10 - 20	0.2	0.3
	20 - 30	0.2	0.2
		9.4	2.6
NMON[a]	0 - 10	53.9	14.0
	10 - 20	6.3	3.5
	20 - 30	0.0	0.2
		60.2	17.7
Total Soil N	0 - 10	64.0	16.4
	10 - 20	7.3	4.4
	20 - 30	0.6	1.2
		71.9	22.0
Total Recovery		81.5	50.3

Summary of ANOVA

Source of Variation	Shoot N	Root N	Mineral N	Microbial N	NMON	Total Soil N
Treatment (T)	†	*	*	***	***	***
Depth (Dp)		***	ns	***	***	***
T x Dp		*	**	***	***	***

[a] NMON, non-microbial organic N

Table IV.4. Absolute and Proportional Microbial Biomass Size and Activity in Glucose Treated (GT) and Non-Glucose Treated (NGT) Soil (0-10 cm) (means of three replicates)[a]

Variable	GT	NGT
Pool Sizes and ^{15}N Recovery		
Absolute		
Microbial C (μg C g^{-1} soil)	626a	521a
Microbial N (μg N g^{-1} soil)	78a	68a
Microbial ^{15}N (% recovery)	9.0a	2.1b
NMO^{15}N (% recovery)	53.9a	14.0b
Proportional		
Microbial C (% soil C)	0.95a	0.79a
Microbial N (% soil N)	1.4a	1.3a
Microbial ^{15}N (% soil ^{15}N)	14.1a	13.0a
NMO^{15}N (% soil ^{15}N)	84.3a	84.5a
NMO^{15}N: Microbial ^{15}N	6.0a	6.7a
C Activity		
Absolute		
CO_2-C evolved (mg g^{-1} soil 10 days^{-1})	0.207a	0.134b
Proportional		
CO_2-C evolved (mg g^{-1} soil C 10 days^{-1})	3.12a	2.03b
CO_2-C evolved (g mg^{-1} microbial C 10 days^{-1})	350a	260a
N Activity		
Absolute		
Net N mineralized (μg g^{-1} soil 10 days^{-1})	16.3a	9.7b
Net ^{15}N mineralized (μg g^{-1} soil 10 days^{-1})	0.083a	0.025b
Proportional		
Net N mineralized (mg g^{-1} soil N 10 days^{-1})	2.88a	1.84a
Net N mineralized (mg g^{-1} microbial N 10 days^{-1})	220a	150a
Net ^{15}N mineralized (mg g^{-1} soil ^{15}N 10 days^{-1})	29.8a	34.2a

[a] Same lowercase letter indicates no significant difference between treatments ($P \leq 0.05$)

Microbial Size and Activity in the 0-10 cm Interval

Generally, microbial activity is most intense in surface soil. Pool sizes and net N mineralization can be expressed on a soil mass basis (absolute) or on a soil C or soil N basis (as a proportion of C or N), but the latter is a better estimate of the availability of soil organic matter to soil microorganisms (Chapter 2 and 3).

There was a tendency for microbial C and N (absolute and proportional) to be greater in GT soil, however the means were not significantly different. Microbial ^{15}N and non-microbial organic ^{15}N (% recovery) were greater in GT soil than in NGT soil (Table IV.4). The proportions of soil ^{15}N which were present as microbial ^{15}N and non-microbial organic ^{15}N were not significantly different between treatments.

Mineralization of C and N were used as indexes of activity of microbial biomass in soil. CO_2-C evolution was greater from GT than NGT during incubation of soil when expressed on a soil mass basis or as a proportion of soil C. There was no significant treatment effect for CO_2-C evolution per unit microbial C in soil. Net N mineralization and net ^{15}N mineralization, expressed per unit soil mass, were greater in GT soil than in NGT soil. Net N mineralization, as a proportion of soil N or microbial N, did not show a treatment effect in soil. Net ^{15}N mineralization showed no treatment effect when expressed as a proportion of soil ^{15}N.

Discussion

This experiment was conducted to determine how manipulation of microbial activity in the field affects the overall availability, distribution and recovery of fertilizer N in a barley-soil system. Microbial activity resulting from glucose addition had a marked effect on N partitioning and barley growth. The high C:N ratio of glucose C and urea N amendments (20:1), and the quantity of glucose added, caused net N immobilization and formation of microbial materials. Recovery of fertilizer ^{15}N within the plant-soil system was 82% in GT and 50% in NGT. In a field experiment Kowalenko (1981) studied the effect of C amendments on N transformations and transport in a fallowed clay loam. Glucose plus fertilizer N addition (4 mg C g^{-1} soil, C:N =59) resulted in a 93% recovery of fertilizer N in 0-75 cm soil, while recovery in the soil not treated with glucose was only 61%, however the difference between treatments was not significantly different. Glucose treatment resulted in a significantly greater recovery of fertilizer N in the surface layers of the soil (Kowalenko 1981). This was also found in the present study.

Microbial uptake and transformation of applied N is rapid early in the season (Jansson and Persson 1982), while the N uptake by barley is greatest about 50 days from seeding in Alberta (Soon 1988). Thus microbes may transform fertilizer N early in the season and affect N partitioning. In a concurrent study, fertilizer N applied without glucose addition was partitioned by late July between shoots (41%), roots (2%), mineral N (5%), microbial N (4%) and non-microbial organic N (16%). Potential losses of N from the soil-plant system by leaching and denitrification were high because 1986 was an exceptionally wet year (Chapter 3).

Shoot mass, root mass and root N in NGT were 1.8-fold, 1.9-fold and 2.2-fold greater, respectively, than in GT. A larger proportion of roots were below the 10 cm depth in GT than in NGT, which may have been related to low levels of available N caused by microbial immobilization in the surface layer. This was reflected in the 3.0-fold greater shoot ^{15}N recovery in NGT plots compared to GT plots. Also, a larger proportion of shoot N consisted of fertilizer N in NGT plants (approximately 16%) compared to GT plants (approximately 10%). In both treatments most soil ^{15}N remaining at harvest was present as non-microbial organic ^{15}N (NMO^{15}N), but recovery of NMO^{15}N was 3.4-fold greater in GT soil than in NGT soil. Thus, microbial response to glucose addition effectively conserved fertilizer N in the active N phase and prevented loss by leaching and denitrification; however, significant remineralization during the season from this phase did not occur to meet plant N demand.

In a greenhouse experiment, Schnürer and Rosswall (1987) found barley shoot mass in a glucose plus N amended soil to be the same as in a soil amended only with N. However, the N application was split and most N was not added at the time of glucose addition, thereby reducing the potential for immobilization.

The legacy of glucose addition had an effect on the microbial biomass in the 0-10 cm interval at harvest time. Microbial ^{15}N recovery 124 days after C addition was greater in GT soil (9%) than in NGT soil (2%), but microbial C and microbial N (absolute and proportional) were not significantly different between treatments, though GT means tended to be greater (Table IV.4). Microbial C was greater in glucose amended soils than controls in short-term (14 to 42 days) laboratory studies (Sparling et al. 1981, Voroney and Paul 1984). Microbial C and N would have been greater in GT immediately after glucose amendment, but this effect was short-lived and not present at harvest.

Glucose addition increased the absolute size of microbial ^{15}N and NMO^{15}N pools in the 0-10 cm interval but did not affect the ratio of these pools in both treatments (Table IV.4). This implies that partitioning of added N between these pools by microbial transformations was similar. This suggests that in the long-term, microbial transformations in soil result in a constant ratio of NMON formed per unit of microbial N formed, and this ratio is not affected by C amendments.

Glucose addition did not have an effect on the proportion of soil C and N which was contained within the microbial biomass, and on CO_2-C evolution and net N mineralization per unit microbial C or N. Therefore the relative pool sizes and net mineralization rate constants were not different between treatments. However, CO_2-C evolution, net N mineralization and net ^{15}N mineralization (per g soil), which are a combination of rate constants and pool sizes, were greater in GT than from NGT during laboratory incubation. It seems that the effect of microbial activity due to glucose addition was still present after one field season.

The results of this study showed that the initial activity of microbes in presence of glucose reduced the availability of N to plants, conserved N in the soil, reduced losses of N from the soil-plant system, and decreased crop yields. Net N mineralization was greater in soil sampled at the end of the season which leads one to think that long-term manipulation of active soil N pools is possible. The potential of this technique may lie in immobilizing fall applied fertilizer to reduce over winter losses of N (Malhi and Nyborg 1983, Mahli et al. 1989). Further investigations are necessary to evaluate the effects of timing of application, rates of glucose plus fertilizer N application, and C/N ratio of amendments on net N mineralization and plant N uptake.

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Chapter 5. Influence of Soil Texture on Habitable Pore Space and Bacterial-Protozoan Interactions in Soil

Introduction

Elliott et al. (1980) investigated the effects of habitable pore space on bacteria-protozoa-nematode interactions in a microcosm experiment. They found, in part, that amoeba grazed bacteria more effectively in a sandy loam subsoil than in a clay subsoil. This was characterized by higher amoebal numbers and a greater increase in CO_2-C evolution in the sandy loam. They thought that amoeba grazed bacteria more efficiently in the sandy loam because it had a greater habitable pore space, although it was not directly measured in the experiment.

More recently, Postma (1989) investigated the effect of habitable pore space on *Rhizobium* numbers in a loamy sand soil and in a silty loam soil. They estimated habitable pore space from the moisture retention curves of the soils. Habitable pore space was varied by changing the moisture content or bulk density in the soils. They concluded that in general, pore space was not limiting to the proliferation and growth of soil microorganisms since total bacterial numbers in a non-sterilized soil did not vary with moisture content or bulk density. Bacterial activity and predator populations were not explicitly investigated. *Rhizobium* populations decreased with increasing moisture contents in the non-sterile soils. Perhaps the reduction of *Rhizobium* numbers in the non-sterilized soils was partially due to soil fauna preferentially feeding on these bacteria, rather than the total soil bacterial population. The authors, however, thought that abiotic factors may have been responsible for the decrease of *Rhizobium* numbers.

Gupta and Germida (1989), studying the protozoa-induced mineralization of bacterial sulfur, found that amoeba in a loamy sand soil reduced bacterial numbers more than in a sandy clay loam soil. The rate of CO_2-C production in the loamy sand increased more than in sandy clay loam when protozoa were added. They thought that a higher number of large pores in the loamy sand soil could explain the larger grazer effect compared to the sandy clay loam soil. However, the pore size distributions were not measured in these soils.

Pore size distribution may be determined by image analysis or by camera lucida (Darbyshire et al. 1985, 1989). Effective pore size distribution may be estimated from the soil moisture retention curve by the capillary rise equation (Hattori and Hattori 1976, Papendick and Campbell 1981, Elliott et al. 1984). Papendick and Campbell (1981) showed that the proportion of pores equal or less than 3 μm were 72% for clay, 40% for silt and 22% for sandy soils. Fine-textured soils generally have a larger proportion of small pores than coarse-textured soils.

Habitable pore space accounts for a small portion of total pore space and affects bacterial and faunal distribution in soils. Microorganisms and small fauna require water films, hence most decomposition of organic matter occurs within water-filled pores (Bamforth 1988). Bacteria probably have the most favorable environment in aggregates with small diameter pores. These pores retain water longer in a drying soil and may offer better protection from faunal predators than larger pores and aggregate surfaces (Hattori and Hattori 1976, Vargas and Hattori 1986, Foster 1988). Up to 60% of soil bacteria pass through a 0.8 μm membrane filter (Bakken and Olsen 1987). Since there is approximately a 3:1 ratio between the diameter of pores and the diameter of bacteria found in them (Kilbertus 1980), a large proportion of bacteria may occupy pores less than 2.4 μm diameter. This small pore size may restrict predation of bacteria by some fauna, such as nematodes and large ciliates. However, some small protozoa can occupy pores down to 2 μm diameter (Bamforth 1985), potentially allowing them to be effective predators of many bacteria. Bacteria contained within pores less than 2 μm would be 'protected' from protozoan predation. More research is needed to elucidate the effects of pore space on bacterial population size and grazer activity (Elliott et al. 1980).

The objective of this study was to test the hypothesis that bacteria are more protected from protozoa predation in fine-textured soils than in coarse-textured soils because they have a larger volume of protected pore space available to them. Therefore, bacterial-feeding protozoa are less effective at reducing bacterial numbers in fine-textured soils versus coarse-textured soils.

Materials and Methods

The Ap horizons of three Orthic Black Chernozemic soils were sampled from the Edmonton area and the samples were stored at 4°C until use. These soils had been in cereal production for at least 10 years. Selected soil properties are presented in Table V.1. Soil samples were taken from the 0-20 cm depth after cereal harvest but prior to disking of the residues. Soils were sieved (2 mm) field moist and then were dried to -1.5 MPa matric potential.

All soils had fine granular structure after sieving, but Soil C also had a large number of non-aggregated sand particles within the matrix. Stereo-microscopic examination showed that the sand grains in Soil C were coated with fine minerals and organic matter, similar to those found in another coarse-textured Black Chernozem from central Alberta (Sanborn and Pawluk 1989). Subsamples of the three soils were adjusted to the bulk densities given in Table V.1 prior to pressure plate determination of water retention. Effective pore size distribution was predicted from the formula $d = 4\sigma / \psi_m$ where d is effective pore diameter (µm), σ is surface tension (7.27×10^{-2} MPa µm at 20°C) and ψ_m is suction (MPa) (Papendick and Campbell 1981). Soil was weighed (100 g moist soil) into 150 mL autoclavable plastic cup assemblies. The cups were designed to allow gas exchange from soil at

Table V.1. Selected Chemical and Physical[a] Properties of the Three Soils

Soil	pH[b]	% C	%N	% sand	% silt	% clay	texture[a]	BD (g cm^{-3})
A	6.7	8.19	0.62	9	46	45	SiC	0.85
B	6.8	5.14	0.42	37	31	32	CL	0.95
C	6.5	2.60	0.31	74	9	17	SL	1.02

[a] particle size was done by the hydrometer method

[b] 1:2 soil:water

the top and bottom of each cup without microbial contamination. Cup assemblies were sterilized by autoclaving (one hour at 121°C and 1.4 kg m^{-2}) three times over a five-day period. Matric potential of the three soils after autoclaving was approximately -1.0 MPa (~0.3 μm water-filled pore diameter).

General Experimental Design: The experiment consisted of 3 soils, 2 treatments (protozoa-plus, protozoa-minus), and 5 major sampling dates. Two minor sampling dates preceeded the major sampling dates in order to obtain some initial measurements. Soil A and B treatments were replicated four times for each sampling date and Soil C treatments were replicated twice.

Inoculation of Bacteria into Sterile Soils: Preliminary work showed that a fluorescent *Pseudomonas* species was common to the three soils. This organism was isolated on 0.1 strength tryptone soya agar and then cultured for one day in Prescott-James medium (Page 1976) with a mixture of micronutrients (designated PJM) containing 0.2 % peptone and 0.2% glucose (pH 7). The cultured bacteria were on average 0.75 μm in diameter and 2.2 μm in length. The culture was centifuged for 15 minutes at 7000 g and washed once in PJM before inoculation into the sterilized soils using a syringe (1 x 10^7 CFU g^{-1} O.D. soil). The soils were then gently mixed and packed to the appropriate bulk densities (Table V.1). Soil matric potential was -0.2 MPa (~1.5 μm water-filled pore diameter) after bacterial inoculation.

Soil Amendment: Glucose-C and KNO_3-N were added to the soils at rates of 360 µg C g^{-1} soil and 30 µg N g^{-1} soil, respectively, four days after bacterial inoculation into sterile soils. The soils were then gently mixed and packed to the appropriate bulk densities (Table V.1). Soil matric potential was -0.05 MPa (~5.8 µm water-filled pore diameter) after C and N amendments. The cup assemblies were placed into disinfected 1.1 L glass jars and incubated in darkness at 22°C. Soil was aerated periodically. The day of glucose and nitrate addition was designated as Day 0.

Protozoa Inoculation: Preliminary work showed that an *Acanthamoeba* species (Lousier and Bamforth 1990) was common to the three soils. This small amoeba was approximately 10 µm in diameter when observed on a microscope slide, but it was able to pass under its own locomotion through polycarbonate Nucleopore filters of 3 µm diameter, but not of 2 µm diameter or smaller. The amoeba was isolated and then cultured for five days on water agar plates wetted with PJM. *Pseudomonas* bacterium served as the amoebal food source. On Day 2, the amoebae were washed from the surface of the agar plates and inoculated into the soils using a syringe (900 amoebae g^{-1} O.D. soil). The protozoa inoculation added negligible numbers of bacteria to the soils. PJM was added to protozoa-minus soils (controls). The soils were then gently mixed and packed to the appropriate bulk densities (Table V.1). Cup assemblies were returned to the glass jars and incubated in a dark room at 22°C. Soil matric potential was -0.03 MPa (~9.7 µm water-filled pore diameter) after protozoa inoculation, which corresponded to 57%, 48% and 36% of water-holding capacity for Soil A, Soil B and Soil C, respectively.

Sampling: Microcosms from each soil type were destructively sampled on Day 0, 2, 4, 7, 12, 21 and 37. Bacteria enumerations were made from Day 0 onwards, except on Day 12. Protozoa enumerations were made from Day 4 onwards.

At sampling the cup assemblies were removed from the mason jars. The soil from each cup was transferred to a sterile beaker and mixed before subsamples were removed for bacterial plate counts, and protozoa MPN determination, as described below. Soil moisture measurements determined that moisture loss during the experiment was negligible.

Bacterial Plate Counts: Twenty grams of moist soil were added to a sterile 250 mL Erlenmeyer flask containing fifteen 2 mm glass beads and 90 mL of PJM. The flask was mixed on a shaker (220 rpm) for 10 minutes and then 10 mL of the suspension was removed to make a 10-fold dilution series (to 10^{-7}) in phosphate saline. Three replicate microcosms were used for plate counts for Soil A and Soil B. Two mL aliquots of the soil suspension from each microcosm were also transferred to two 20 mL test tubes for protozoa determination. Triplicate aliquots (0.1 mL) of diluted soil suspensions were spread on each of three 0.1 strength tryptone soy agar plates. The plates were inverted and incubated for five days at 22°C before counting. Fungal contamination checks were made on potato dextrose agar plates containing 120 ppm streptomycin.

Protozoa MPN Determination: Protozoa were determined using the microtiter plate MPN method of Darbyshire et al. (1974) with modifications. One of the two 2-mL soil suspensions obtained from each microcosm soil (see above) was diluted to 6 mL with PJM. The other 2 mL suspension (heat-treatment) was placed in a 45°C water bath for 18.5 minutes before being diluted to 6 mL with PJM. Heat treatment kills active protozoa but allows cyst forms to survive (K. Zwart, personal communication). The soil suspensions were vortexed for 5 seconds and then placed into a sterile reservoir. An 8-tip 25 μL microdiluter was immediately dipped into the soil suspension and then placed into the first row of wells on the microtiter dish. One hundred μL of PJM (with 0.01% peptone) was previously added to each of the microtiter wells. The microdiluter was then used to dilute 5-fold from the first to the eleventh row of wells. All wells were then inoculated with 25 μL of *Pseudomonas* which served as a food source for the protozoa. Bacteria were cultured for a day before use in PJM (with 0.2% peptone and 0.2% glucose). They were then washed by centrifugation at 5000 rpm and diluted 8- to 10-fold in PJM with 0.01% peptone before addition. The twelfth row of wells served as a check for bacterial culture contamination or PJM contamination. The culture wells were incubated one week in the dark at 15°C and then screened using an inverted microscope at 200-400x magnification. The culture wells were then incubated for one week at 22°C before negative wells were rescreened. The MPN of total protozoa (no heat treatment) and of encysted protozoa (heat treatment) were calculated by using a microcomputer program developed by Clark and Owens (1983).

Statistical Analyses: Analyses of variance was performed on experimental variables using the SAS package GLM. Preliminary analysis determined that bacteria and protozoa numbers were non-normal with heterogenous variance, thus log 10 transformations were performed on the data before ANOVA. The GLM statement LSMEANS was used to differentiate treatment means within each soil and date.

Results

Pore Size Distribution

The initial effective pore size distributions of the three soils showed that the porosity of Soil A (65%) was greatest and Soil C (60%) the least (Fig. V.1). Pores with < 0.75 μm effective diameter made up 26.0 %, 19.7% and 10.9% of the soil volume for Soil A, Soil B and Soil C, respectively. Pores between 0.75 μm and 2.0 μm effective diameter made up 4.3 %, 3.1% and 2.4% of the soil volume for Soil A, Soil B and Soil C, respectively. It was assumed that bacteria contained within this pore size range were protected from protozoa predation. Pores between 2.0 and 9.7 μm effective diameter made up 7.0 %, 5.6% and 3.4% of the soil volume for Soil A, Soil B and Soil C, respectively. It was assumed that protozoa would inhabit pores within this range. The ratio of 'non-protected' pore volume (2.0-9.7 μm effective diameter) to 'protected' pore volume (0.75-2.0 μm effective diameter) was 1.6, 1.8 and 1.4 for Soil A, Soil B and Soil C, respectively.

Bacterial Numbers

On Day 0 bacterial numbers per g soil were greatest in Soil A and least in Soil C (Fig. V.2, Table V.2). In Soil B and Soil C the addition of glucose, nitrogen and protozoa resulted in an increase of bacteria until Day 4. On Day 4 bacterial numbers in all three soils were approximately 3×10^9 g^{-1} soil. After Day 4 the protozoa-plus treatment had significantly lower bacteria than the protozoa-minus treatment in all 3 soils (Fig. V.2, Table V.2). During this time bacteria declined in all soils and in both treatments. The greatest reduction of bacteria due to

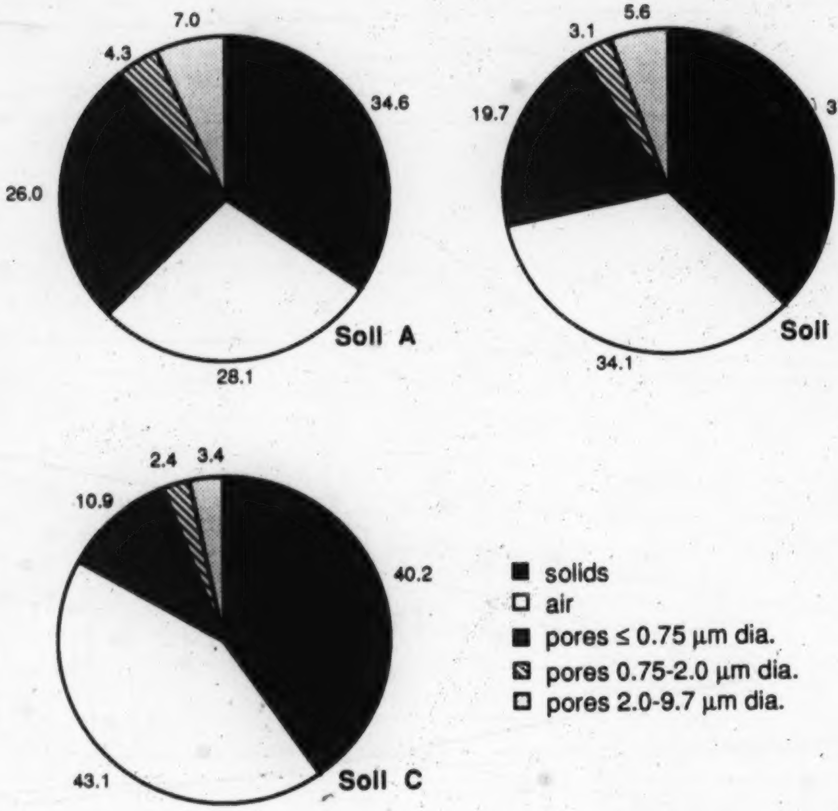

Figure V.1. Soil Volume (%) Occupied by Solids, Air and Water-filled Pores in the Three Soils When Soil Matric Potential Equalled -0.03 MPa

protozoan grazing occurred between Day 4 and Day 7. Compared to the protozoa-minus treatment, bacteria in the protozoa-plus treatment were reduced by 68%, 50%, and 75% in Soil A, Soil B and Soil C, respectively.

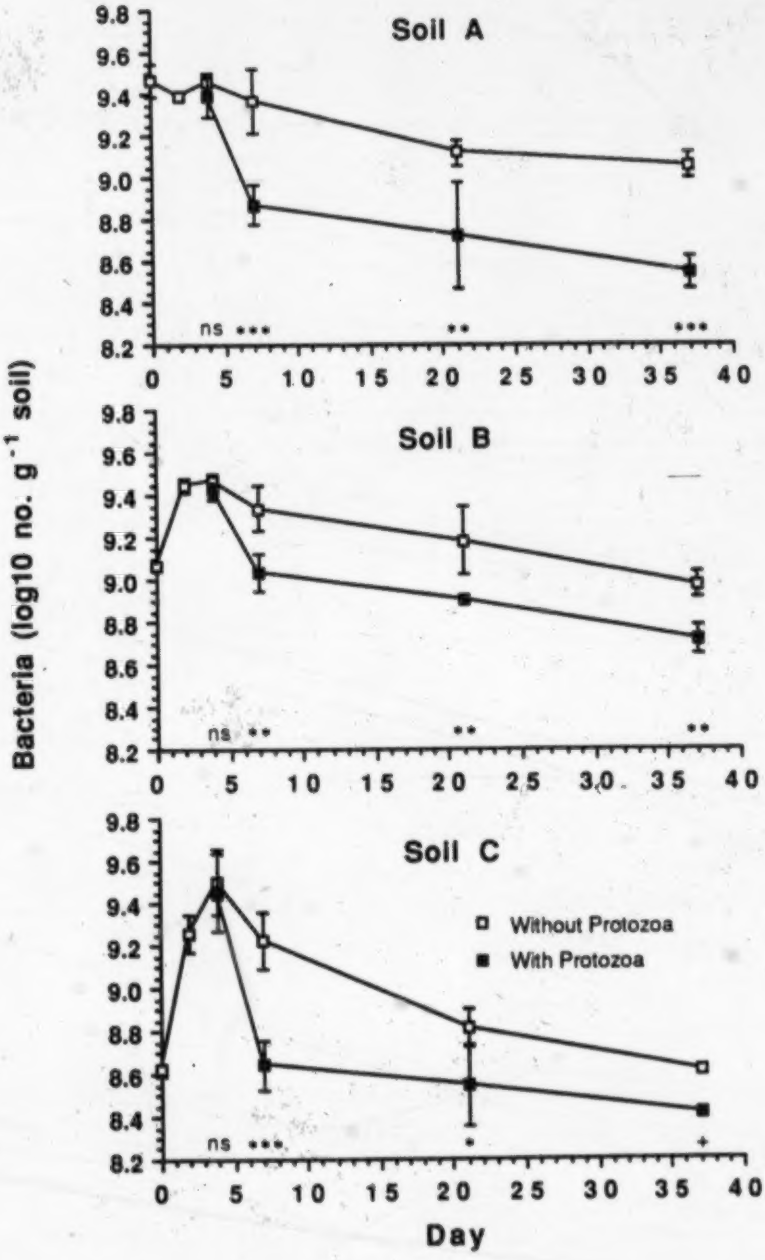

Figure V.2. Bacterial Numbers in the Presence and Absence of Protozoa in the Three Soils (the difference between treatment means within a soil and day is significant at: +, $p \leq 0.10$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; vertical bars represent ± 1 standard error)

Table V.2. Summary of ANOVA[a] for Bacterial Numbers (log10 no. g^{-1} soil)

Source of Variation	Bacteria
Soil (S)	***
Treatment (T)	***
S x T	†
Day (D)	***
S x D	**
T x D	***
S x T x D	*

In this and the following table:

[a] The difference between means is significant at: †, p≤0.10; *, p≤0.05; **, p≤0.01; ***, p≤0.001; ns, not significant

Protozoa Numbers

Protozoa numbers followed similar trends in all three soils (Fig. V.3, Table V.3). The difference between total protozoa (non-heat treatment) and cystic protozoa (heat treatment) was active protozoa. On Day 4, two days after protozoa inoculation, protozoa were all active. Numbers were 10330, 4760 and 15380 g^{-1} soil for Soil A, Soil B and Soil C, respectively. Between Day 4 and Day 7, the period of greatest bacterial decline, total protozoa increased greatly to 150480, 96160, and 192100 g^{-1} soil for the three soils, respectively; however, most protozoa became cystic by Day 7. Active protozoa gradually increased between Day 12 and Day 21, although the difference between total protozoa and cystic protozoa often was not significant due to the variability of the data. The sensitivity of the MPN method was not great enough to make an accurate estimate of active protozoa numbers after Day 4. Total and cystic protozoa in Soil B and Soil C declined greatly after Day 21, but protozoa in Soil A declined only slightly.

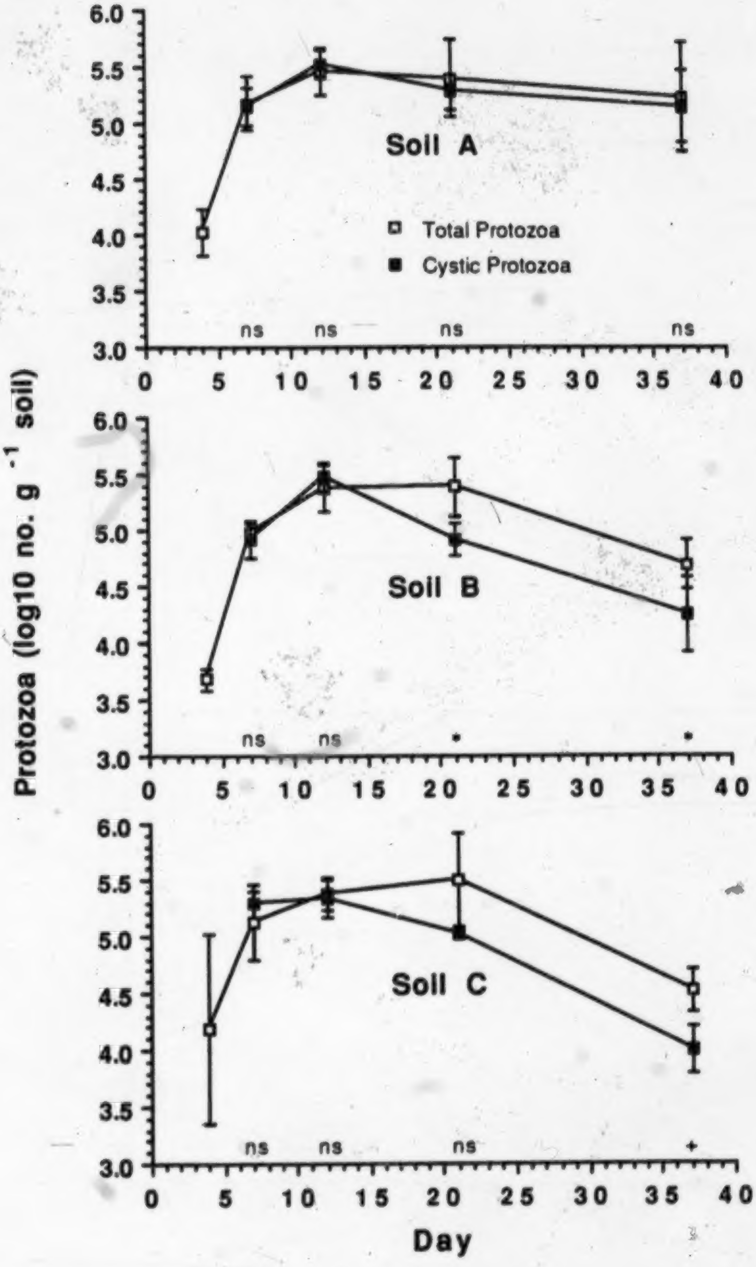

Figure V.3. Total and Cystic Protozoa Numbers in the Three Soils (see Fig. V.2 for levels of significance)

Table V.3. Summary of ANOVA for Protozoa Numbers (log10 no. g^{-1} soil)

Source of Variation	Protozoa
Soil (S)	***
Heat /Non Heat Treatment (HNH)	*
S x HNH	ns
Day (D)	***
S x D	**
HNH x D	†
S x HNH x D	ns

Discussion

Impact of Soil Texture on Bacterial Protection From Protozoan Predation

Habitable pore spaces of the three soils were estimated at the beginning of the experiment by using soil moisture retention curves and determining the effective pore size diameters of the pores by the capillary rise equation. These estimations of pore size distribution were only approximate because of hysteresis effects, irregular pore shapes (Hattori and Hattori 1976, Elliott et al. 1984, Postma and van Veen, submitted) and due to the slight shrink-swell characteristics of the soil clays. Nevertheless, the estimations allowed relative comparisons of the restrictions which the soil matrix might impose upon organism distribution and movement. The soils were gently mixed during amendment addition and during inoculations. The mixed soils were adjusted to the initial bulk densities. Hence, it was assumed that manipulation of the soils changed pore size distributions only slightly.

Soils were at approximately -1.0 MPa matric potential at the time of bacterial inoculation. This corresponded to a maximum water-filled pore diameter of 0.3 µm. This matric potential was chosen so that bacteria would be drawn into the smaller pores by matric suction when the concentrated bacterial suspension was inoculated into the soils (Postma et al. 1989). Theoretically, the smallest pores which the bacteria should be drawn into would be 0.75 µm in diameter, the diameter of the bacteria, although most bacteria are found in pores 2-3 times their diameter (Kilbertus 1980). No attempt was made to determine the actual distribution of bacteria in the soils after inoculation. However, it can be assumed that some bacterial penetration into aggregates occurred since Vargas and Hattori (1986) found that 50% of *Aerobacter* introduced into a sterile sandy clay loam soil was contained within aggregate pores < 2.5 µm effective pore diameter. It was assumed that the distribution of bacteria would not be the same as in non-sterilized soil where soil bacteria are intimately mixed with the soil matrix by cultivation and natural soil processes.

The *Acanthamoeba* species was able to pass through a 3.0 µm diameter polycarbonate filter, but not a 2.0 µm diameter filter when grown in minimal media containing *Pseudomonas*. Its small size should make it an effective bacterial predator in small pore spaces. The lower limit of porosity available for protozoan inhabitation, 2.0 µm diameter pores, was approximately the preferred pore size for the bacteria (Kilbertus 1980). It was inferred that pores with diameters between 0.75 µm and 2.0 µm would be available only to bacteria, hence this was taken to represent the 'protected' pore space for bacteria. Pores within this size range occupied almost twice as great a volume in Soil A (4.3% of soil volume) as in Soil C (2.4% of soil volume). Because bacteria and protozoa live in water-films (Bamforth 1985) it was assumed that the maximum pore size that these organisms could occupy was less than 9.7 µm diameter, the maximum water-filled pore diameter. Pores larger than 9.7 µm diameter were not filled with water. Pores between 2.0 µm and 9.7 µm diameter would therefore be accessible to both bacteria and protozoa and thus were 'unprotected'. Pores within this size range occupied approximately twice as great a volume in Soil A (7.0% of soil volume) as in Soil C (3.4% of soil volume). Thus, the 'protected' and 'non-protected' pore volumes were both greatest in Soil A.

The *Acanthamoeba* species effectively grazed the *Pseudomonas* in all soils, as shown by the reduced bacterial numbers. Reduced bacterial numbers are common when protozoa are added to microcosms (Kuikman and van Veen 1989, Elliott et al. 1979, Heynen et al. 1988, Gupta and Germida 1989). Most bacteria were accessible to predation during the initial period of the incubation. At this time bacterial numbers were high and many bacteria would have been in large pores that were accessible to protozoa. The greatest effects of the protozoa occurred rapidly, and early in the experiment. Between Day 4 and Day 7 protozoa reduced bacterial numbers greatly when compared to soil containing only bacteria. Over the same time period, protozoa numbers increased greatly in the soils. By Day 7, bacteria numbers were low and most protozoa encysted. Protozoan encystment may have been due to the lack of an easily obtainable food source. Bacterial numbers do not necessarily correlate with bacterial production, due to the possiblity of increased bacterial turnover (Fenchel and Harrison 1976); however, the reduction of bacteria was so great (up to 75%) that it is possible that increased bacterial production could not support an active protozoa population as large as the total protozoa numbers observed on Day 7.

Protozoa were associated with a greater reduction of bacteria in the coarse-textured soil. Even though all soils showed reduced bacterial numbers due to grazing, the coarse-textured soil, showed the largest decrease between Day 4 and Day 7. After Day 7, bacterial numbers in the protozoa-plus treatment declined slightly in Soil C but numbers in Soil A and Soil B continued to decline at a greater rate. Protozoa in Soil C may have preyed upon relatively more bacteria than the protozoa in the fine-textured soils, and they also may have grazed bacteria more rapidly than protozoa in the fine-textured soils.

Assuming that the reduction of bacterial numbers was due to protozoan grazing, bacterial consumption rates of the protozoa in the various soils can be calculated by dividing the difference between bacterial numbers in the grazed and ungrazed microcosms by the number of protozoa in the soil. This method is approximate because bacterial growth rates are assumed to be the same in both treatments and bacteria are assumed not to regrow and replace the consumed cells (Gupta and Germida 1989). On Day 7, the consumption rates of the protozoa in Soil A, Soil B and Soil C were 11400, 7300 and 15200 bacteria per protozoa, respectively. The more rapid consumption rate by protozoa in Soil C could be an ecological advantage since much terrestrial bacterial activity in nature occurs in bursts, usually after wetting events (Clarholm 1989). The bacterial consumption rates were higher than those reported by Kuikman and van Veen (1989), but were in the same range as those reported by Bryant et al. (1982), and Gupta and Germida (1989).

The actual distribution of bacteria within the pores of the three soils was not measured, therefore one can only speculate about the reason(s) why the bacteria were not as accessible to protozoa in the fine-textured soils as in the coarse-textured soil. Several theories are put forward: (i) the porosity available for protozoan habitation in Soil C was one half the volume of that in Soil A. Soil microorganisms typically occupy only 0.17% of soil organic matter surfaces and 0.02% of soil mineral surfaces (Hissett and Gray 1976). Protozoa located within the non-protected pore volume in Soil C would have had smaller distances to travel than in the other soils when searching for bacterial prey. Feeding rates of protozoa have been shown to be dependent on the concentration of the bacterial food source

(Alexander 1981). Also, the pores in the fine-textured soils may have been longer and more tortuous than in the coarse-textured soil, making it more time and energy consuming for the protozoa to find their prey; (ii) a larger proportion of soil bacteria may have been located within the 'protected' pore volume in Soil A and Soil B, than in Soil C. The 'protected' porosity was twice as great in Soil A as in Soil C. Perhaps a larger proportion of the total soil bacteria in the fine-textured soils found suitable refuge within this pore range, compared to the coarse-textured soil; (iii) the physical location of the 'protected' pores may have been a factor. In fine-textured soils most of the small pores are present within soil aggregates. Many bacteria contained within the small pores of the aggregates would be separated, relatively speaking, by a large distance from pores large enough to contain protozoa. In Soil C, stereo microscopic examination showed that many of the sand grains appeared to be coated with fine mineral particles and soil organic matter. Sanborn and Pawluk (1989) also found coated sand particles in a coarse-textured Black Chernozem from central Alberta. Aggregations of primary particles smaller than sand were not as common in Soil C as in the other two soils. Therefore, many of the small pores in Soil C were probably located within coatings around sand particles. Bacteria reproducing within pores of these coatings would be in close proximity to larger pores containing protozoa, thereby increasing the likelihood of predation.

Bacterial numbers in the presence of protozoa in the three soils were significantly lower than those in the controls. Also, there was a greater reduction of bacteria in the coarse-textured soil. Therefore, these results support the hypothesis that bacteria are more protected from protozoan predation in fine-textured soil than in coarse-textured soil. The physical environment of the soils affected the interaction of bacteria and protozoa, therefore this has to be considered when calculating the turnover of C and N in soils..

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Chapter 6. Influence of Soil Texture on Protozoa-Induced Mineralization of Bacterial C and N

Introduction

In arable soils free-living protozoa regulate the structure of the bacterial community and influence short-term nutrient cycling (Clarholm 1984, Bamforth 1985). Microcosm studies have shown that microbial turnover rates and mineralization are often increased when microbial predators are present (Anderson et al. 1981, Clarholm 1985, Coleman et al. 1984, Griffiths 1986, Kuikman and van Veen 1989a). Faunal grazing leads to a loss of C as CO_2-C and a surplus of N which is excreted as NH_4-N (Coleman et al. 1984). This process may be especially important under conditions of N limitation since N mineralized by protozoa may make NH_4-N available to microbes and plants (Anderson et al. 1981, Clarholm 1985, Robinson et al. 1989) thereby decreasing the limitation on the decomposition rate of high C/N ratio substrates.

Turnover of C and N within the microbial biomass is faster in coarse-textured soils than in fine-textured soils (van Veen et al. 1984, 1985; Merckx et al. 1985). Textural effects may impose restrictions on the ability of fauna to graze on microbes (Chapter 5), therefore texture may play a role in faunal-induced mineralization of microbial C and N. Elliott et al. (1980) found, in part, that amoeba grazed bacteria more in a sandy loam subsoil than in a clay subsoil. This was characterized by higher amoebal numbers and a greater increase in CO_2-C evolution in the sandy loam. The effect of texture on N mineralization was not investigated. Gupta and Germida (1989), studying the protozoa-induced mineralization of bacterial sulfur, found that amoeba in a loamy sand soil reduced bacterial numbers more than in a sandy clay loam soil. The rate of CO_2-C production in the loamy sand increased more than in sandy clay loam when protozoa were added. They thought that a higher number of large pores in the loamy sand soil could explain the larger grazer effect compared to the sandy clay loam soil.

In Chapter 2 and 3 it was found that C and N mineralization during laboratory incubation, expressed as a proportion of microbial C or N, was greater in a Gray Luvisol than in a Black Chernozem. Though not significant, protozoa numbers prior to incubation were much greater in the Gray Luvisol than in the Black Chernozem. It is possible that the protozoa in the coarser-textured Luvisol grazed more bacteria than in the finer-textured Chernozem. This may have partly contributed to the different mineralization rates in the two soils. Few studies have reported the effects of soil texture on bacterial-protozoan interactions in soil, including C and N mineralization. An integrated study is needed to measure the effects of soil texture on dynamics of bacteria, C and N mineralization in the presence and absence of predators.

The objective of this study was to test the hypothesis that protozoa grazing on bacteria would increase the mineralization of bacterial C and N, and this effect would be most apparent in coarse-textured soils. This study was conducted concurrently with the study described in Chapter 5.

Materials and Methods

The properties of the three Black Chernozemic soils used are described in Chapter 5 and Table V.1. Soil was weighed (100 g moist soil) into 150 mL autoclavable plastic cup assemblies. The cups were designed to allow gas exchange from soil at the top and bottom of each cup without microbial contamination. Cup assemblies with soil were sterilized by autoclaving (one hour at 121°C and 140 kPa) three times over a five-day period.

General Experimental Design: The experiment consisted of 3 soils, 2 treatments (protozoa-plus, protozoa-minus), and 5 major sampling dates. Two minor sampling dates preceeded the major sampling dates to obtain some initial measurements. Soil A and B treatments were replicated four times for each sampling date and Soil C treatments were replicated twice.

Inoculation of Bacteria into Sterile Soils: The cultured *Pseudomonas* species was inoculated (1 x 10^7 CFU g^{-1} O.D. soil) into the three soils. The soils were gently mixed and packed to the appropriate bulk densities as described in Chapter 5.

Labelling of Bacterial Biomass: The bacterial population was labelled *in situ* with ^{14}C and ^{15}N prior to protozoa inoculation. Glucose-^{14}C (specific activity=13 kBq mg^{-1} C) and KNO_3-^{15}N (excess=4.55%) were added to the soils at rates of 360 μg C g^{-1} soil and 30 μg N g^{-1} soil, respectively, four days after bacterial inoculation into sterile soils. This relatively small amendment rate was used to prevent the formation of large quantities of non-microbial organic-C and -N in the soils (Chapter 4). The soils were then gently mixed and packed to the appropriate bulk densities (Table V.1). The cup assemblies were placed into disinfected 1.1 L glass jars containing a CO_2-C trap (20 mL of 0.5 M NaOH). Each cup assembly-jar unit was referred to as a microcosm. Glass jars containing NaOH served as CO_2 blanks. Microcosms and CO_2 blanks were incubated in darkness at 22°C. The day of glucose and KNO_3 addition was designated as Day 0.

Although the soils contained NH_4-N and NO_3-N before glucose-^{14}C addition, KNO_3-^{15}N was added on Day 0 to label the bacterial biomass and follow the N dynamics. Bacterial growth on glucose would cause immobilization of KNO_3-^{15}N. Subsequent mineralization would result in the release of NH_4-^{15}N. Since nitrifiers were not present in the autoclaved soil, the NH_4-^{15}N would not be further transformed into NO_3-^{15}N. The accumulated NH_4-^{15}N would be due to bacterial mineralization and/or amoebal mineralization. Therefore the dynamics of three ^{15}N pools were studied: (i) NO_3-^{15}N which remained in the soil and was not taken up by bacterial biomass; (ii) NH_4-^{15}N which originated from the bacterial biomass and was mineralized during bacterial turnover and protozoan grazing; and (iii) organic-^{15}N in soil that was mainly generated by the microbial biomass or its by-products.

Protozoa Inoculation: The cultured *Acanthamoeba* species was inoculated (900 amoebae g^{-1} O.D. soil) into the three soils on Day 2, as described in Chapter 5. The soils were then gently mixed and packed to the appropriate bulk densities (Table V.1). Cup assemblies were returned to the glass jars and incubated in a dark room at 22°C. CO_2-C blanks were aerated and then incubated with the microcosms. Soil matric potential was -0.03 MPa after protozoa inoculation.

Sampling: Microcosms from each soil type were destructively sampled on Day 0, 2, 4, 7, 12, 21 and 37. Mineral N and bacteria population measurements were taken from Day 0 onwards. CO_2-C, CO_2-^{14}C, total soil ^{14}C, total soil ^{15}N and mineral ^{15}N measurements were taken from Day 2 onwards. Protozoa number measurements were taken from Day 4 onwards. Bacterial populations were not measured on Day 12. No C or N measurements were obtained for the protozoa-plus treatment from Soil C on Day 37.

At sampling the cup assemblies and NaOH containers were removed from the glass jars. The soil from each cup was transferred to a sterile beaker and mixed before subsamples were removed for bacterial plate counts, and protozoa MPN determination (see Chapter 5). The remaining soil was air dried and ground for total soil C, total soil ^{14}C, total soil N and total soil ^{15}N analyses. Soil moisture measurements determined that moisture loss during the experiment was negligible.

Bacterial and Protozoa Enumerations: These were enumerated as described in Chapter 5.

Soil Respiration: Five mL and 0.5 mL aliquots of the 0.5 M NaOH were used for analysis. The 5 mL aliquot was titrated with 0.1 N HCl to determine the CO_2-C evolved. The 0.5 mL aliquot was mixed with 12 mL of Packard HIONIC® fluor in a 20 mL scintillation vial. ^{14}C was determined by counting for 20 minutes in a Packard Tri-Carb 2000 CA scintillation counter.

Soil C and N Analyses: Total C was determined on ground soil by dry combustion using a Leco Carbon Determinator CR-12. The quantity of ^{14}C in 100 mg soil was determined after oxidation in a Harvey Biological Oxidizer, Model OX300. ^{14}C released during oxidation was trapped in Harvey's ^{14}C cocktail and counted for 20 minutes in a Packard Tri-Carb 2000CA scintillation counter. Recovery of ^{14}C as CO_2 plus soil C was generally greater than 90%.

Soil mineral N was extracted by mixing 20 g soil with 100 mL 2 M KCl soil solution for one hour and then filtering through Whatman No. 42 filter paper. The extract was frozen before analysis. Technicon Autoanalyzers were used to measure NO_3-N (Industrial Method No. 497-77A, 1977) and NH_4-N (Industrial Method No. 98-70W, 1973).

Total soil N and all ^{15}N analysis were performed after dry combustion using a V. G. Isogas ANA-SIRA 10 system. ^{15}N analysis was performed on NO_3-N and NH_4-N obtained by microdiffusion technique (Brooks et al. 1989). NH_4-N was obtained after the addition of MgO to convert NH_4-N to NH_3-N which diffused out to a glass fiber filter disk containing 2 N $KHSO_4$ to trap NH_3-N as NH_4-N. Containers were kept sealed for 6 days to allow complete diffusion of the NH_3-N out of solution. Devardas alloy was then added to convert NO_3-N to NH_3-N, another filter disk with 2M $KHSO_4$ was placed over the solution and the containers were left sealed for 6 days.

Statistical Analyses: Analyses of variance was performed on all experimental variables using the SAS package GLM. The GLM statement LSMEANS was used to differentiate treatment means within each soil and date.

Results

Bacterial and Protozoa Numbers

Bacterial and protozoan dynamics have been described in detail in Chapter 5 (Fig. V.2, Fig. V.2, Table V.2 and Table V.3). On Day 4 bacterial numbers in all three soils were approximately 3 x 10^9 g^{-1} soil. After Day 4 the protozoa reduced bacterial numbers in all 3 soils. The greatest reduction of bacteria due to protozoan grazing occurred between Day 4 and Day 7 in Soil C.

Protozoa numbers increased greatly to as much as 192000 g^{-1} soil five days after inoculation. Soil C had the greatest total protozoa numbers early in the experiment. In all soils most protozoa became cystic on Day 7, however the proportion of total protozoa which were active gradually increased after Day 12.

CO_2-C Evolution

In all soils the addition of protozoa significantly increased CO_2-C evolution per g soil (Fig. VI.1, Table VI.1) relative to the protozoa-minus treatment. The greatest change upon protozoa addition occurred between Day 4 and Day 7. On Day 7, protozoa-plus treatments were greater than protozoa-minus treatments by

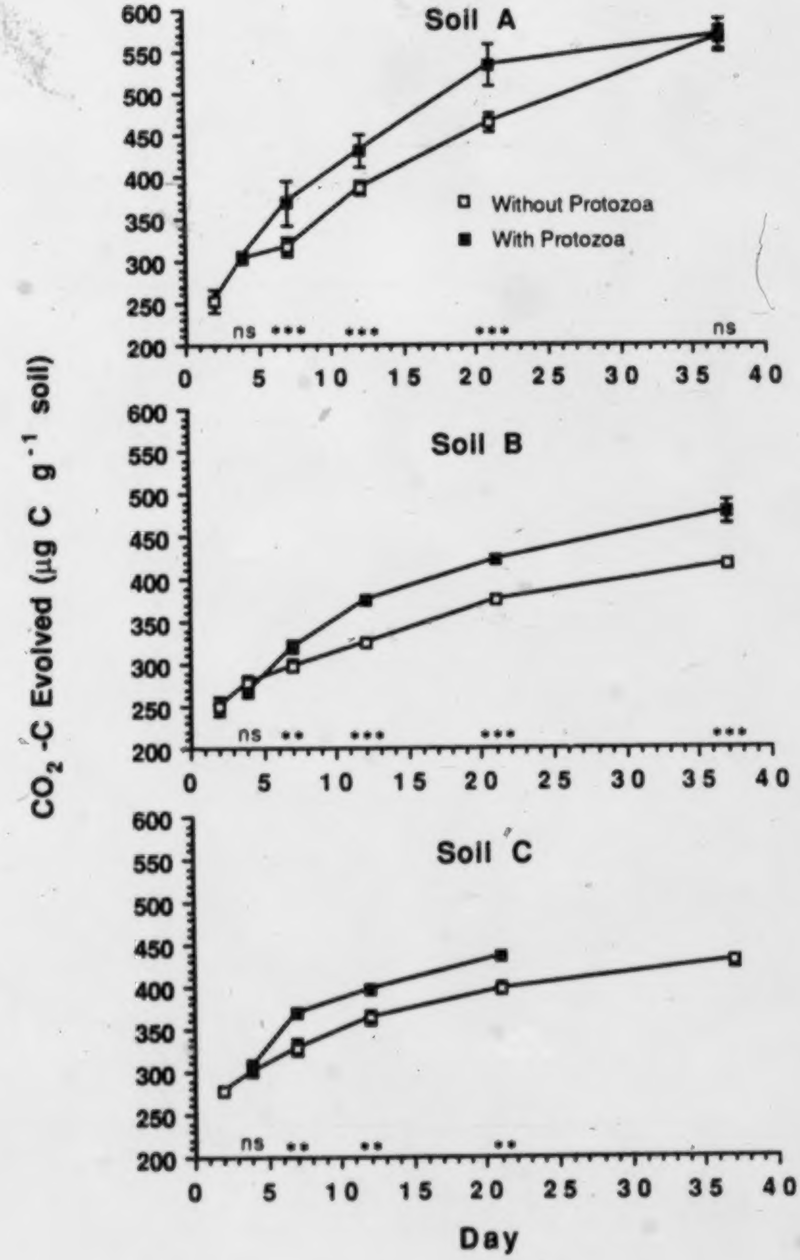

Figure VI.1. CO_2-C Evolution in the Presence and Absence of Protozoa from the Three Soils (in this and subsequent figures the difference between treatment means within a soil and day is significant at: +, $p \leq 0.10$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; vertical bars represent ± 1 standard error)

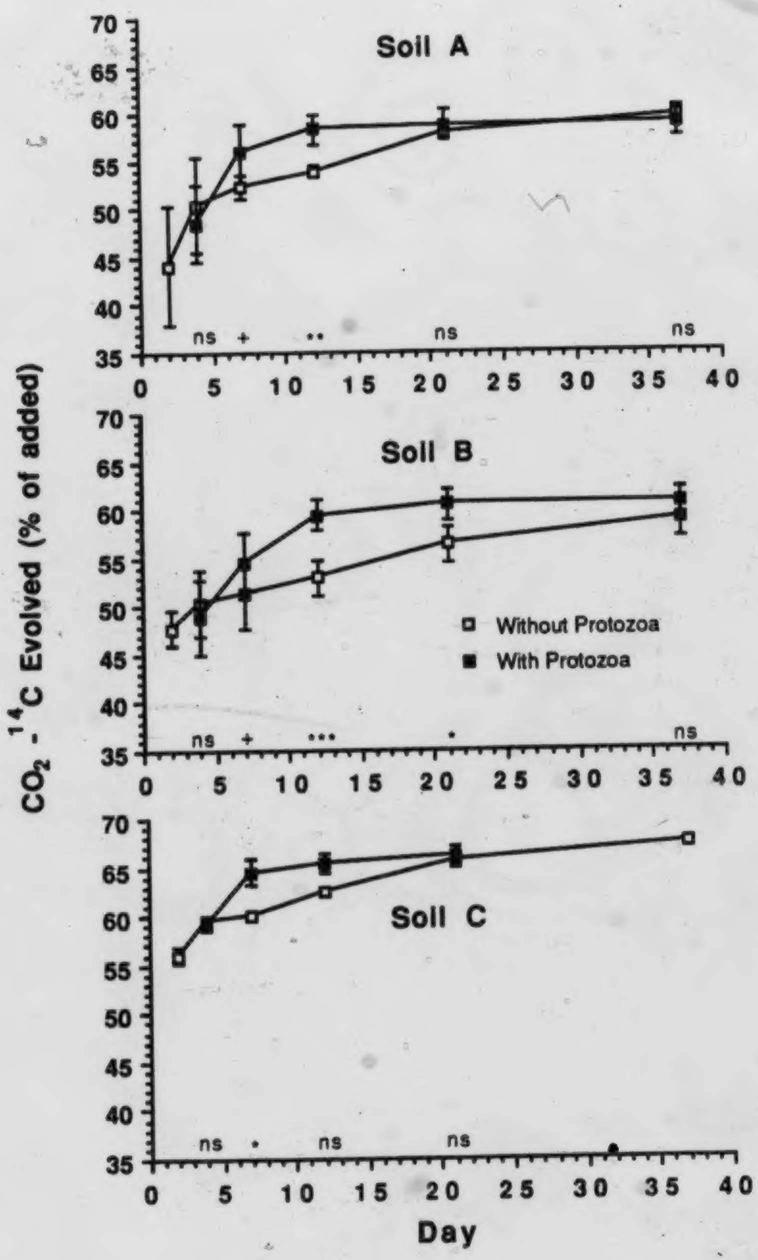

Figure VI.2. CO$_2$-^{14}C Evolution in the Presence and Absence of Protozoa from the Three Soils

Table VI.1. Summary of ANOVA[a] for CO_2-C (mg C 100 g^{-1} soil) and CO_2-^{14}C Evolution (% recovery)

Source of Variation	CO_2-C	CO_2-^{14}C
Soil (S)	***	***
Treatment (T)	***	***
S x T	ns	ns
Day (D)	***	***
S x D	***	ns
T x D	***	**
S x T x D	***	ns

In this and all subsequent tables:

[a] The difference between means is significant at: †, $p \leq 0.10$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ns, not significant

16%, 8% and 12% for Soil A, Soil B and Soil C, respectively. After Day 7 CO_2-C evolution from protozoa-plus soils approximately parallelled the evolution from protozoa-minus soils. In general, CO_2-C evolution from Soil A was greater than from Soil B and Soil C.

CO_2-^{14}C Evolution

In the first two days 44%, 47% and 56% of the glucose ^{14}C added to Soil A, Soil B and Soil C, respectively, was evolved as CO_2-C (Fig. VI.2). Over the incubation period more CO_2-^{14}C was evolved from Soil C than from the other soils. Protozoa quickly increased CO_2-^{14}C evolution from all soils (Fig. VI.2) Table VI.1). CO_2-^{14}C evolution generally increased throughout the incubation in protozoa-minus treatments. In contrast, CO_2-^{14}C evolution from protozoa-plus treatments increased very slightly after Day 7 for Soil C and after Day 12 for Soil A and Soil B (Fig. VI.2). Consequently, the two treatments within each soil evolved between 60 and 67% of CO_2-^{14}C at the end of the incubation.

Between 56 and 44% of ^{14}C-glucose was transformed into bacterial C and their metabolites after 2 days. Protozoa inoculation decreased bacterial numbers rapidly between Day 4 and 7, thus the effect of water and C amendment, and

Table VI.2. Summary of ANOVA for NO_3-N and NH_4-N (µg N g^{-1} soil)

Source of Variation	NO_3-N	NH_4-N
Soil (S)	***	***
Treatment (T)	ns	***
S x T	ns	*
Day (D)	***	***
S x D	†	†
T x D	ns	***
S x T x D	ns	ns

inoculation lasted up to Day 12. Less than 3% additional ^{14}C was evolved in the protozoa-plus treatments between Day 12 and Day 37. The latter time interval represents organism activity in moist unperturbed soils. The ^{14}C remaining in soil at Day 37 was taken to be present as bacterial and amoebal C and newly formed organic matter.

Mineral N Concentration

On Day 0, NO_3-N (Fig. VI.3, Table VI.2) was greatest in Soil C (61 µg g^{-1} soil) and least in Soil A (44 µg g^{-1} soil) after KNO_3 addition (30 µg g^{-1} soil). Amendment addition resulted in net NO_3-N immobilization of 13, 18 and 19 µg N g^{-1} soil, for Soil A, Soil B and Soil C, respectively by Day 2. NO_3-N in Soil A and Soil B decreased slightly until Day 7 and then became relatively stable. After Day 21 NO_3-N decreased slightly in all soils. NO_3-N was not significantly different between the two treatments.

On Day 0, NH_4-N concentration (Fig. VI.4, Table VI.2) was greatest in Soil A (50 µg g^{-1} soil) and least in Soil B (34 µg g-1 soil). Amendment addition resulted in net NH_4-N immobilization of 17, 12 and 23 µg N g^{-1} soil for Soil A, Soil B and Soil C, respectively, by Day 2. Net mineralization of NH_4-N occurred from Day 2 to Day 21 in all soils and treatments, although the protozoa-minus

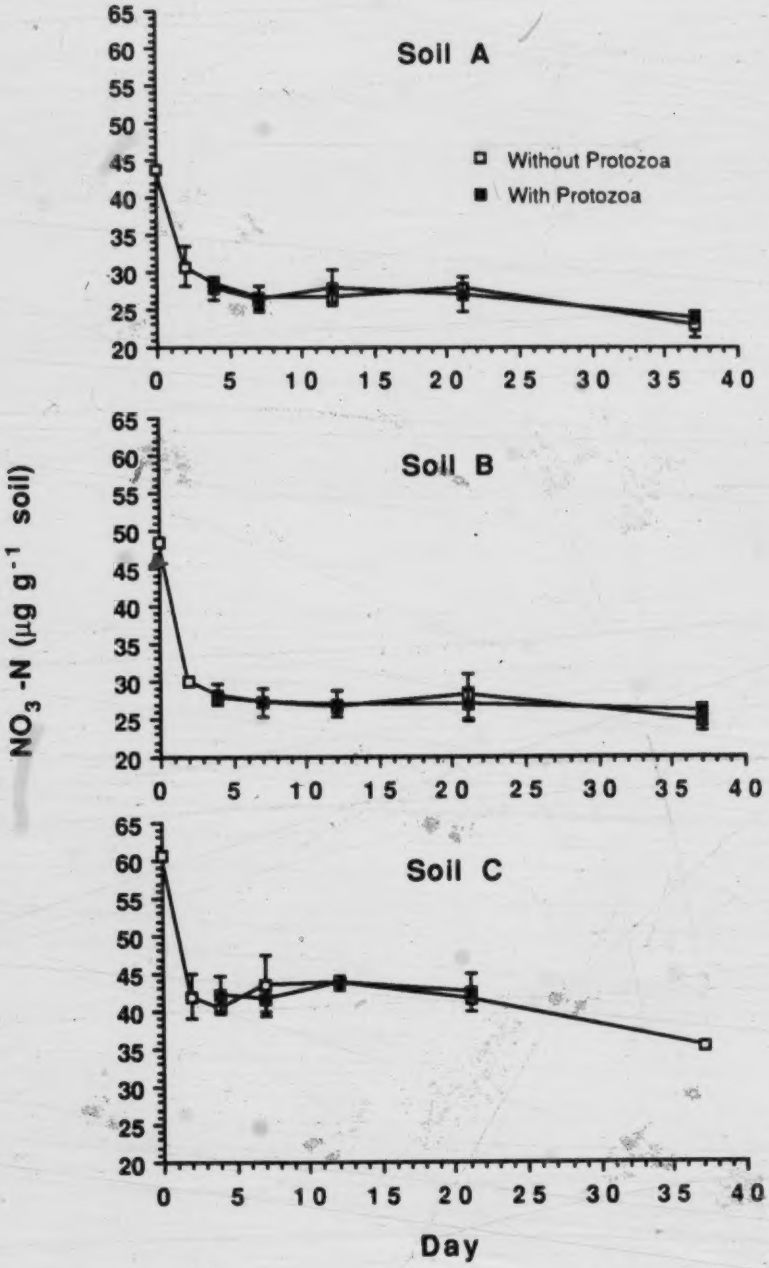

Figure VI.3. NO_3-N Concentration in the Presence and Absence of Protozoa in the Three Soils (treatment means were not significantly different)

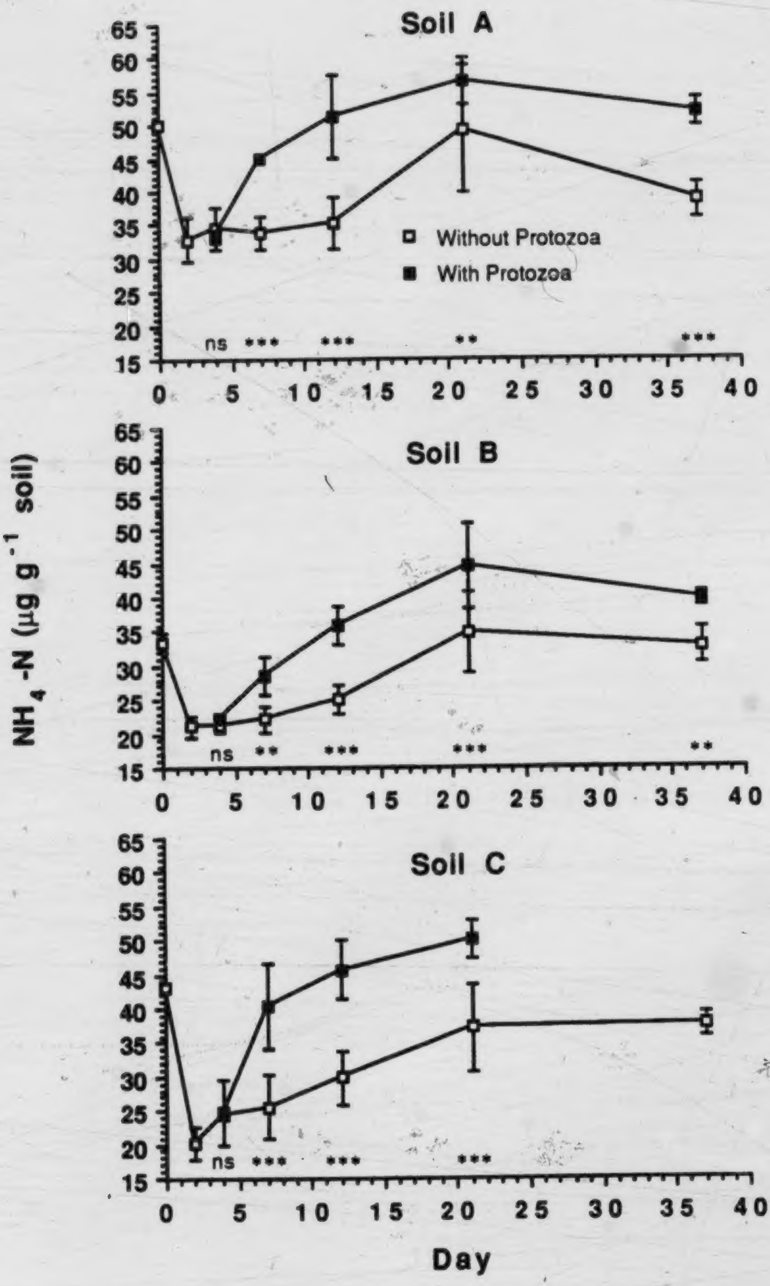

Figure VI.4. NH_4-N Concentration in the Presence and Absence of Protozoa in the Three Soils

Table VI.3. Summary of ANOVA for NO_3-^{15}N, NH_4-^{15}N, and Organic-^{15}N Recovery (% of added ^{15}N)

Source of Variation	NO_3-^{15}N	NH_4-^{15}N	Organic-^{15}N
Soil (S)	***	***	***
Treatment (T)	ns	***	**
S x T	ns	***	ns
Day (D)	***	***	*
S x D	†	***	ns
T x D	ns	***	ns
S x T x D	ns	**	ns

treatment in Soil A did not increase much until Day 12. After Day 21 NH_4-N concentration was stable or decreased slightly.

Protozoa addition significantly increased net NH_4-N mineralization in all three soils. The greatest increase occurred between Day 4 and Day 7. On Day 7, protozoa-plus treatment concentrations exceeded protozoa-minus concentrations by 33%, 29% and 58% for Soil A, Soil B and Soil C, respectively.

Partitioning of KNO_3-^{15}N

On Day 2, two days after amendment, ^{15}N recovered as NO_3-^{15}N (Fig. VI.5, Table VI.3) was 49%, 46% and 62% for Soil A, Soil B and Soil C, respectively. Between Day 4 and Day 7 NO_3-^{15}N decreased slightly in Soil A and Soil B, and in the protozoa-plus treatment of Soil C. After Day 7, recoveries varied slightly with time, except for the protozoa-minus treatment of Soil C which gradually decreased until Day 37.

On Day 2, ^{15}N recovered as NH_4-^{15}N (Fig. VI.6, Table VI.3) was 2.1%, 3.4% and 2.9% for Soil A, Soil B and Soil C, respectively. Recovery of NH_4-^{15}N generally increased with time, although the protozoa-minus treatment in Soil A did not increase until after Day 12. NH_4-^{15}N either did not change, or it decreased

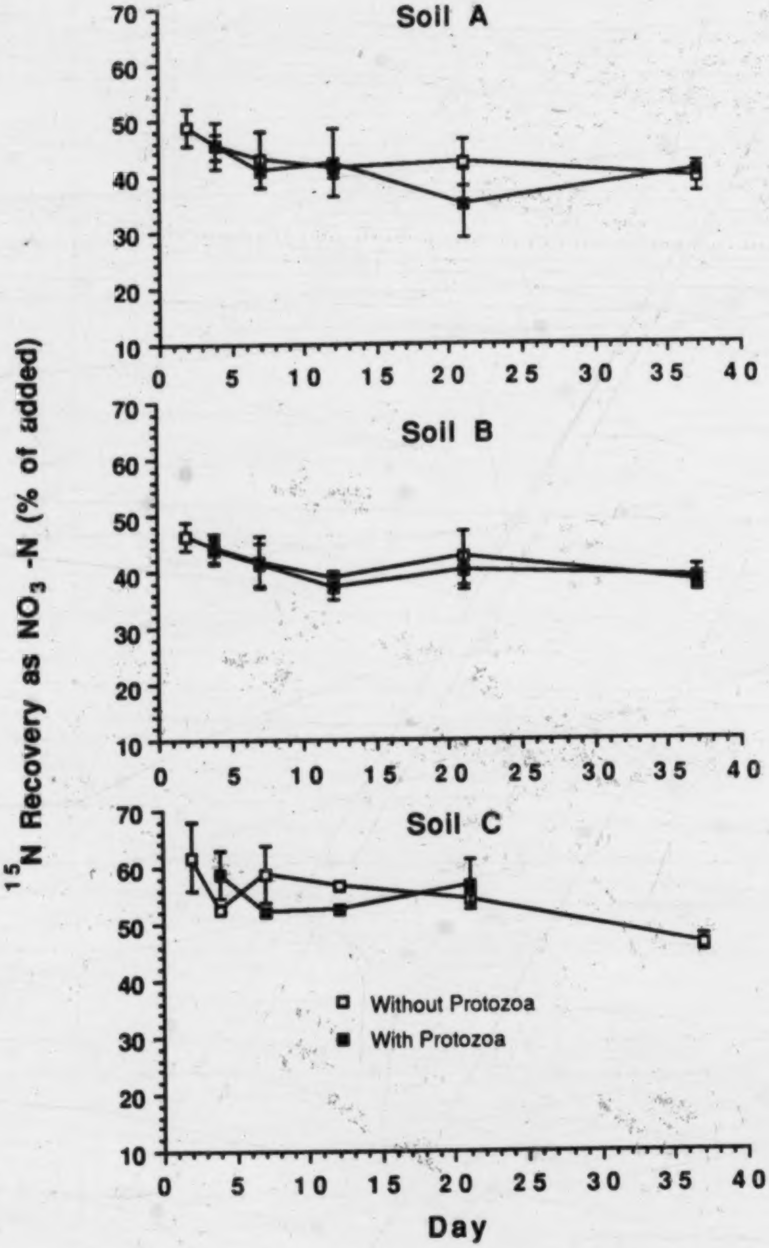

Figure VI.5. NO$_3$-^{15}N Recovery in the Presence and Absence of Protozoa in the Three Soils (treatment means were not significantly different)

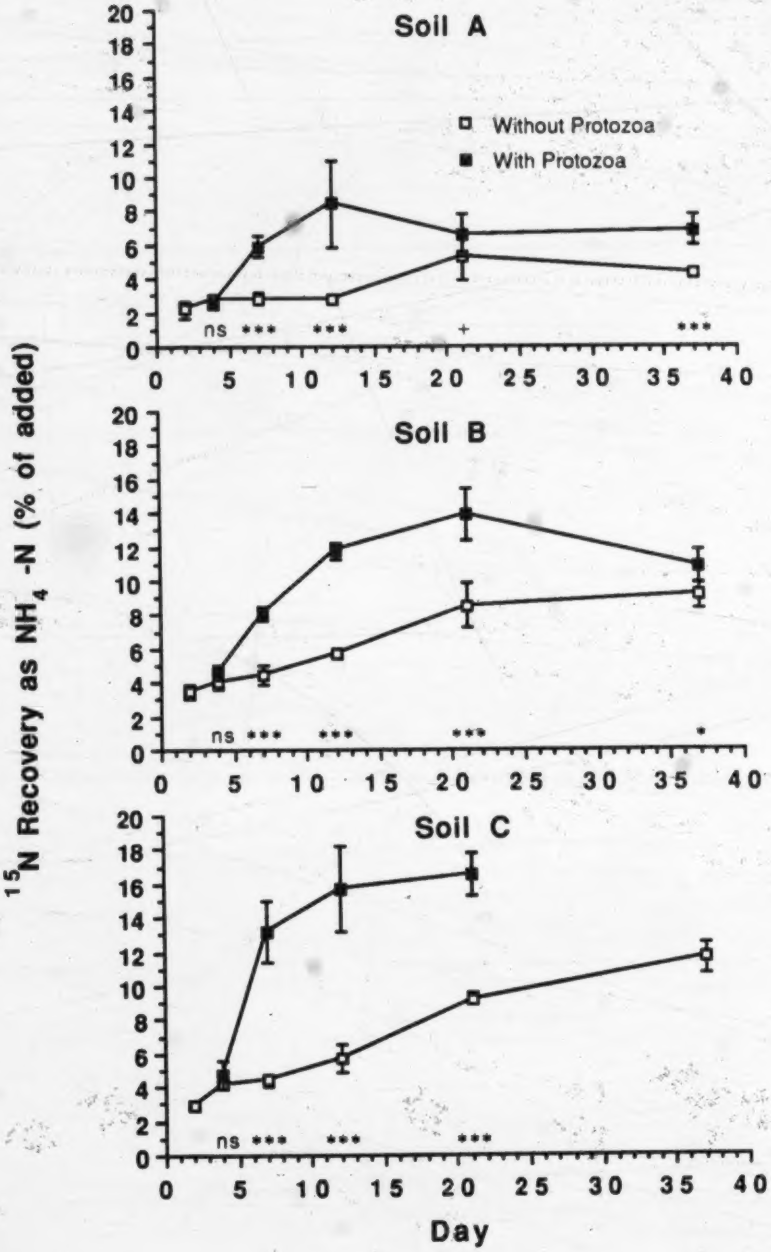

Figure VI.6. NH_4-^{15}N Recovery in the Presence and Absence of Protozoa in the Three Soils

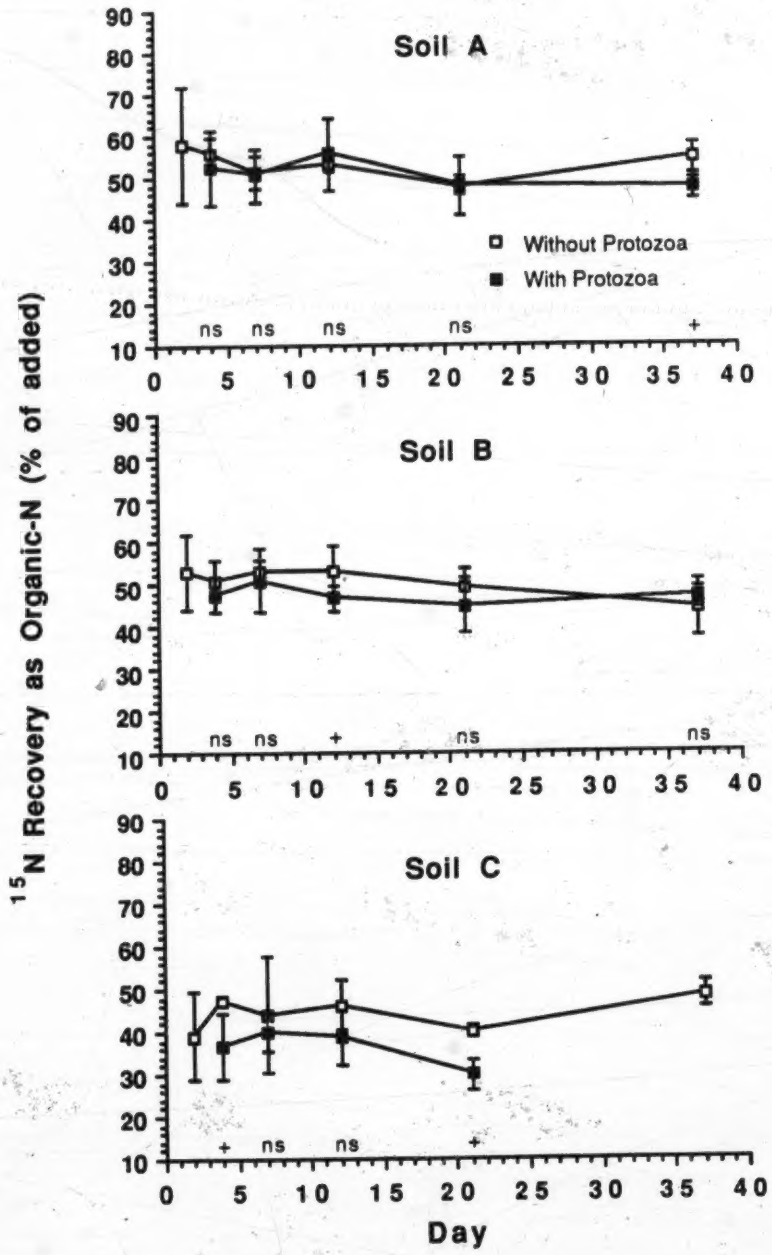

Figure VI.7. Organic ^{15}N Recovery in the Presence and Absence of Protozoa in the Three Soils

slightly after Day 21 in Soil A and Soil B. NH_4-^{15}N in Soil C increased throughout the measurement period.

In all soils the addition of protozoa significantly increased net NH_4-^{15}N mineralization relative to the protozoa-minus treatment. The greatest rate of net NH_4-^{15}N mineralization occurred between Day 4 and Day 7. On Day 7, mineralization from protozoa-plus treatment exceeded protozoa-minus treatment by 110%, 80% and 200% in Soil A, Soil B and Soil C, respectively.

The non-mineral ^{15}N remaining in the soils was assumed to be in the microorganisms, or their by-products, hence this pool was called organic ^{15}N. Organic ^{15}N (Fig. VI.7, Table VI.3) was greatest in Soil A and least in Soil C. In Soil B and Soil C the protozoa-plus treatment had significantly lower recoveries of organic ^{15}N than the protozoa minus treatment on some sampling dates.

Discussion

The results of this study will be discussed for two time periods: (i) a period of rapid changes between Day 0 and Day 12 due to inoculation of organisms and amendment of C and N to soils, and (ii) a period of slower changes from Day 12 to Day 37.

Effect of Texture on Dynamics of Carbon and Nitrogen During the First 12 days after Amendment Addition

Even though all soils showed reduced bacterial numbers due to grazing, the coarse-textured soil showed the largest decrease between Day 4 and Day 7, the period of greatest protozoa number increases (Chapter 5). After Day 7, bacterial numbers in the protozoa-plus treatment declined slightly in Soil C but numbers in Soil A and Soil B continued to decline at a greater rate. Protozoa in Soil C may have preyed upon relatively more bacteria than the protozoa in the fine-textured soils, and they also may have grazed bacteria more rapidly than protozoa in the fine-textured soils. On Day 7, the consumption rates of the protozoa in Soil A, Soil B and Soil C were 11400, 7300 and 15200 bacteria per protozoa, respectively (Chapter 5).

CO_2-C evolution in all protozoa-plus soils increased greatly over the controls between Day 4 and Day 7, reflecting increased grazer activity. This supports the findings of other researchers (Gupta and Germida 1989, Kuikman et al. 1990, Coleman et al. 1984). The rate of CO_2-C evolution was very similar between treatments after Day 7, indicating that protozoa had a marginal effect at increasing C mineralization after this time.

The CO_2-^{14}C results showed differences that confirmed a texture effect for C originating from the bacterial biomass. All soils showed increases of CO_2-^{14}C evolution due to grazing but the evolution from Soil C reached a maximum by Day 7, compared to Day 12 for Soil A and Soil B. This parallelled the bacterial dynamics where Soil C numbers were greatly reduced by the protozoa and did not decrease much after Day 7. Hence a portion of the immobilized ^{14}C was non-mineralizable and protozoa contributed to the quick depletion of labile soil ^{14}C. The bacteria in the protozoa-minus treatment also utilized labile soil ^{14}C and the CO_2-^{14}C evolved from both treatments was similar late in the incubation. This suggests that a maximum amount of soil ^{14}C is lost as CO_2-^{14}C from each soil and that protozoa affected the rate, but not the quantity of soil ^{14}C which was lost. The amount lost as CO_2-^{14}C was greatest from Soil C and least from Soil A.

The N results provided further support for the hypothesis that protozoan grazing was greater in the coarse-textured soil than in the fine-textured soils. NH_4-N and NO_3-N concentrations decreased as N was immobilized due to the glucose amendment. A total of 30, 30 and 42 μg N g^{-1} soil was immobilized between Day 0 and Day 2 for Soil A, Soil B and Soil C, respectively. The bacteria in Soil C probably immobilized more N because amendment addition caused a greater increase in bacterial numbers than in the other two soils. Mineralization of N as NH_4-N in the protozoa-minus treatment occurred more rapidly in Soil C, indicating that bacterial turnover in this soil had resulted in a larger surplus of N than in the other soils. The mineralization of N is determined by the consumption rate of the substrate, the C/N ratio of the substrate and the bacteria, and the utilization efficiency of the bacteria (Robinson et al. 1989).

When protozoa were present NH_4-N mineralization increased relative to the protozoa-minus treatment in all soils. Increased N mineralization and bacterial N turnover due to the presence of protozoa, with or without the presence of plants, has been reported by many authors (Elliott 1979, Woods et al. 1982, Coleman et al. 1984, Clarholm 1985, Ritz and Griffiths 1987, Couteaux et al. 1988, Kuikman et al. 1989a, 1989b, 1991). Soil C had the greatest percent increase in net NH_4-N mineralization relative to the protozoa-minus treatment. Thus protozoa were more effective at mineralizing the recently immobilized N in the coarse-textured soil.

Less NO_3-^{15}N was immobilized by Day 2 in Soil C than in Soil A and Soil B because Soil C had more non-labelled NH_4-N plus NO_3-N on Day 0, the day of amendment addition. Hence the label was subject to more dilution in Soil C. NO_3-^{15}N remaining in soil did not differ between treatments within each soil and date, indicating that protozoa addition did not affect the immobization of NO_3-^{15}N into the bacterial biomass. NH_4-^{15}N mineralization in the protozoa-minus treatment showed the greatest increase with time in Soil C, compared to the other soils, which also compliments the greater evolution of CO_2-^{14}C in this soil. This supports the findings of van Veen et al. (1984, 1985), Merckx et al. (1985), Dinwoodie and Juma (1988) and in this thesis (Chapter 2 and 3), that turnover of C and N within the microbial biomass is faster in coarse-textured soils than in fine-textured soils.

Even though some organic ^{15}N would have been present as active ^{15}N at the time of protozoa addtion, most ^{15}N at Day 2 would have been present as bacterial ^{15}N (Voroney and Paul 1984). The increased net NH_4-^{15}N mineralization in the presence of protozoa may have originated mainly from bacterial ^{15}N since the experiment was not conducted under conditions of mineral N limitation. Under conditions of N limitation protozoan grazing would increase bacterial metabolic activity (Anderson et al. 1981, Robinson et al. 1989) thereby increasing the decomposition of soil organic matter or plant residues having a high C/N ratio, resulting in the increased mineralization of ^{15}N from the active N phase. The latter concept was not examined in this study.

Protozoa had the greatest effect of increasing net NH_4-^{15}N mineralization in Soil C (Fig. VI.5), therefore protozoa were more effective at mineralizing bacterial N in this soil than in the fine-textured soils. On Day 7 the percent increase in NH_4-^{15}N mineralization, compared to the protozoa-minus treatment, was much greater in Soil C (200%) than in Soil A (110%) and Soil B (80%). The time required to reach the maximum concentration of NH_4-^{15}N was greater than the time needed for maximum CO_2-^{14}C evolution because of the internal cycling which occurs for soil ^{15}N. In contrast, soil ^{14}C is not recycled once it is mineralized to CO_2-^{14}C.

The more effective grazing of bacteria by protozoa in Soil C can also be seen if one considers the proportion of organic-^{15}N which is mineralized to NH_4-^{15}N. If the increase in NH_4-^{15}N between Day 4 and Day 7 is divided by the organic-^{15}N present on Day 4, less than 1% of organic-^{15}N is mineralized in the three protozoa-minus soils; however, 6%, 7% and 23% is mineralized in the protozoa-plus treatment for Soil A, Soil B and Soil C, respectively. Hence, it can be clearly seen that grazing of bacterial N was most effective in Soil C.

Effect of Texture on Dynamics of Carbon and Nitrogen 12 to 37 days after Amendment Addition

The effect of texture on protozoan grazing was not as marked after Day 12. During this time period the influence of amendment addition had ceased and changes in populations and C and N dynamics were reduced. Protozoa had little effect at further reducing bacterial numbers and the majority of amoeba were present as cysts (Chapter 5). CO_2-C evolution continued to increase in all soils but the rate of evolution for each soil was unaffected by the presence of protozoa. CO_2-^{14}C continued to increase in protozoa-minus treatment for each soil but evolution from the protozoa-plus treatment was very slight. NH_4-N and NH_4-^{15}N concentrations in both treatments increased at the same rate or decreased due to net N immobilization.

The influence of protozoa was reduced during the latter part of the experiment because glucose-induced bacterial growth had ceased and protozoa had reduced bacterial numbers greatly during the first 12 days. The ^{14}C and organic-^{15}N remaining in soil was contained within a protected bacterial population, active or inactive protozoa or microbial by-products. Active protozoan grazers could not access much of this ^{14}C or ^{15}N. NH_4-^{15}N continued to increase in both treatments for a longer period of time than CO_2-^{14}C evolution because N cycles internally. The immobilization of mineral N and ^{15}N after Day 21 was probably due to the death of protozoa and bacteria, thereby supplying a readily available C source for the remaining bacterial population.

Implications

The results of this study supported the hypothesis that protozoan-grazing on bacteria increase the mineralization of bacterial C and N; and, that this effect is most apparent in the coarse-textured soils. This effect may partially explain the greater turnover of C and N in coarse-textured soils compared to fine-textured soils. This study also showed that protozoa induced effects are sometimes transient in soil. Kuikman et al. (1990) also found that protozoa only stimulated C turnover during the initial stages of a laboratory incubation. In nature, the greatest effects of protozoa on bacterial turnover and mineralization probably occur briefly after perturbations, such as aggregate disruption, energy addition (Ritz and Griffiths 1987) or water addition (Bryant et al. 1982, Clarholm 1989, Hunt et al. 1989, Kuikman et al. 1991). Thus, protozoa probably are ecologically important in nature during the brief periods following perturbations which stimulate microbial growth. Soil protozoa have rapid encystment-excystment abilities, as was observed in this study, to help survive changes in the soil environment (Bryant et al. 1982, Bamforth 1988).

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Chapter 7. Simulation of Protozoa-Induced Mineralization of Bacterial C and N

Introduction

One of the challenges of soil ecological research is to link the structural characteristics of the biological community to functional processes (Richards 1974). Population ecology is poorly reflected in nutrient cycling research for three reasons: (i) soil population ecology is difficult because of the complexity of the soil system and the lack of discriminating techniques, (ii) in general, population ecologists and biogeochemists have not had mutual interests, and (iii) nutrient cycling research at the ecosystem level is recent and much research has been descriptive (Heal et al. 1989). Before linking dynamics of soil organism populations to nutrient cycling in an ecosystem, specific questions regarding the interaction of organisms amongst themselves and with the soil matrix need to be addressed. Controlled laboratory experiments are useful to accomplish this.

One way to evaluate the linkage between soil populations and soil processes is through simulation modelling. Mathematical description of a complex system such as soil reduces ambiguity and provides a framework for integrating existing information and testing hypotheses (Jeffers 1982). Gaps in knowledge are also identified. Thus models are often more useful to develop and test hypotheses rather than as predictive devices (van Veen et al. 1981, Pomeroy et al. 1988).

A variety of simulation models describing the mineralization and immobilization of N (Frissell and van Veen 1981, Juma and Paul 1981, McGill et al. 1981, Voroney et al. 1981, van Veen et al. 1984, Hunt et al. 1986, Parton et al. 1988) and food webs (Hunt et al. 1984, 1987, 1989; Moore et al. 1990) already exist. Most soil organic N models emphasize microbial biomass. In constrast soil food web models describe the microbial-faunal interactions explicitly but do not describe soil organic matter dynamics adequately. Experiments and results described in chapters 5 and 6 stimulated the development of a new model to describe bacterial-protozoan interactions and their impact on C and N cycling in two soils with different textures. This model integrates the concepts of soil organic matter as described in the TRAMIN model (Juma and Paul 1981) and the bacteria-protozoa component of the food web model (Hunt et al. 1984). The unique features of this model are: (i) it combines the food web with specific soil C and N pools, (ii) it simultaneously traces the flows of C, ^{14}C, N and ^{15}N, and (iii) it accounts for protection of bacteria from protozoan grazing in soils of different texture. The objective of this study was to simulate bacterial-protozoan interactions and flows of C and N in two soils with different textures under laboratory conditions. The model was first adapted to fit data from Soil B (clay loam, Chapter 5 and 6) and then was validated with data from Soil C (sandy loam, Chapter 5 and 6). These two soils represented two extremes of protozoa-induced mineralization of bacterial C and N (Chapter 6). The model was run on a Mac II si® microcomputer using Stella II® software.

Model Description

The model (Figure VII.1) consisted of four submodels: (i) C submodel, (ii) ^{14}C submodel, (iii) N submodel, and (iv) ^{15}N submodel. A complete set of flow diagrams and equations are presented in Appendix 1 and 2.

C and N State Variables

The state variables containing C and N were bacteria, amoeba, soluble, active, and stable organic matter. Additional C state variables were glucose, and bacterial and amoebal CO_2-C. Additional N variables were NO_3-N and NH_4-N.

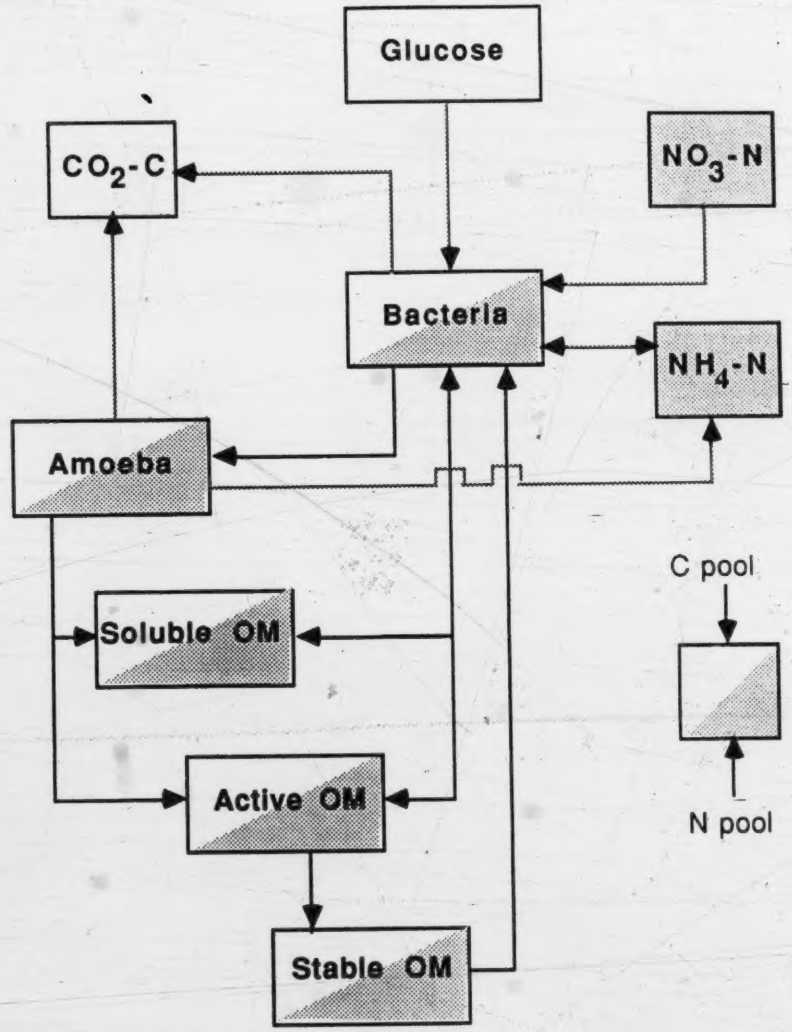
Glucose
CO2-C
NO3-N
Bacteria
NH4-N
Amoeba
C pool
Soluble OM
Active OM
N pool
Stable OM

Figure VII.1. Flow Chart of C and N Transfers in the Simulation Model (solid lines indicate transfer of C and N while dotted lines indicate transfer of C or N, OM refers to organic matter)

Initial pool sizes for Soil B and Soil C are presented in Table VII.1. Bacterial C was estimated from plate counts. The *Pseudomonas* species was measured at the time of inoculation and was estimated to have an average volume of 9.72 x 10^{-1} μm^{-3} . The organism was assumed to have a density of 1.1 g cm^{-3}, a dry weight of 20%, and a C content of 45% (Paul and Clark 1989). The bacterial C/N ratio was initialized to 4.5. *Acanthamoeba* numbers were determined using MPN technique and the amoeba was assumed to have a mass of 1.2 x 10^{-3} µg per individual, a dry weight of 20% and a C content of 50% (Coleman et al. 1978). The C/N ratio of the amoeba was set at 7 (Hunt et al. 1984).

The soluble C and N pools represent small very rapid cycling pools consisting of labile C and N organic materials, such as metabolites and cytoplasmic materials of dead organisms. Soluble C was not measured in the laboratory incubation, however the initial pool size was estimated from previous work on similar soils (Dinwoodie and Juma 1988). The intial C/N ratio of the soluble pool was set to 10.

The active C and N pools represent rapid cycling insoluble organic matter pools, consisting of microbial metabolic products and recently stabilized materials (Paul and Juma 1981). Campbell and Souster (1982) determined the active N fraction of cultivated Black Chernozemic soils from Saskatchewan to be 5.2% and 8.8% of total soil N for a clay loam and a sandy loam, respectively. These percentages were used to calculate the intial pool size of active organic matter in the present model. The initial C/N ratio of the active pools were set to 6 (Juma and Paul 1981).

Fifty percent of soil organic matter consists of chemically recalcitrant material with a half-life of 600 years (Campbell et al. 1967). This phase was not simulated because it would have an insignificant effect on short-term dynamics described in the present model. The remainder of the soil organic C was partitioned into bacteria, amoeba, and soluble, active and stable organic matter. The initial pool size

Table VII.1. Initial (Day 0) C and N Pool Sizes (μg C or N g^{-1} soil) Used in the Model for Soil B (clay loam) and Soil C (sandy loam)

	C Pool Size		N Pool Size	
Pool	Soil B	Soil C	Soil B	Soil C
Glucose	360	360	-	-
Bacteria	111	40	24.7	8.9
Amoeba (Day 2)	1.1	1.1	0.16	0.16
Soluble OM	80	80	8	8
Active OM	1308	1638	218	273
Stable OM	19855	13134	1805	1194
NO_3-N (fertilizer NO_3-N)	-	-	18 (30)	31 (30)
NH_4-N	-	-	34	43

of stable organic matter was calculated by difference. The C/N ratio of the stable pool was initialized to 11 (Juma and Paul 1981).

^{14}C and ^{15}N State Variables

The ^{14}C and ^{15}N submodels were analagous to the corresponding C and N submodels. ^{14}C pools consisted of ^{14}C activity (Bq g^{-1} soil). All ^{14}C pools were initialized to 0, except the glucose ^{14}C pool which was initialized to 4680 Bq g^{-1} soil (360 μg glucose C g^{-1} soil x 13 Bq μg^{-1} glucose C). ^{15}N pools consisted of μg ^{15}N excess g^{-1} soil. All ^{15}N pools were initialized to 0, except the NO_3-^{15}N pool which was initialized to 1370 ng^{-1} ^{15}N g^{-1} soil (30 μg NO_3-N g^{-1} soil x 4.55 % ^{15}N excess x 1000 ng μg^{-1}).

C Flows between State Variables

The flows within the C submodel directly or indirectly controlled the flows within the other submodels. Bacterial uptake (U) (μg C g^{-1} soil day^{-1}) of glucose-C (g) was calculated by a Michaelis Menten equation (Hunt et al 1984):

$$Ug = \frac{Vg \times G \times Bc}{Kg + G} \tag{1}$$

where: Vg = maximal specific glucose uptake (μg C μg^{-1} bacterial C, day^{-1})

G = initial glucose concentration (μg C g^{-1} soil)
Bc = bacterial C (μg C g^{-1} soil)
Kg = half saturation constant for glucose (μg C g^{-1} soil)

Mineral N was abundant during the incubation, therefore it was assumed that glucose uptake was not limited by available N.

Bacterial uptake (Uc) of other C substrates (soluble C , active C and stable C (μg C g^{-1} soil day^{-1})) were calculated independently using first order kinetics (Juma and Paul 1981):

$$Uc = -\frac{dC}{dt} = k \times C \tag{2}$$

where: k = first order rate constant for the specific substrate (day^{-1})
C = concentration of the soil C substrate (μg g^{-1} soil)
t = time (day)

Rate of bacterial CO_2-C evolution (Rb) (μg C g^{-1} soil day^{-1}) was calculated using growth and maintenance components (Hunt et al. 1984):

$$Rb = ((1-YB) \times Uc) + (Bc \times Mb) \tag{3}$$

where: YB = maximum possible yield of bacteria for consumption of specific substrates (unitless)
Uc = bacterial uptake of specific substrates (μg C g^{-1} soil day^{-1})
Mb = bacterial maintenance rate (day^{-1})

Bacteria death rate (Db) was proportional to the amount of bacterial C present:

$$Db = \frac{d(Bc)}{dt} = -Kdb \times Bc \tag{4}$$

where: Kdb = bacterial death rate constant (day^{-1})

Thirty-five percent of this material was assumed to be soluble and entered the soluble C fraction. The remaining 65% was assumed to be insoluble or chemically stable and entered the active C pool.

Protozoa consumption (Pab) of bacteria ($\mu g\ C\ g^{-1}$ soil day^{-1}) was calculated by a Type III sigmoid functional response (Hunt et al. 1984):

$$Pab = \frac{Vab \times Ac}{1 + (Kab/Bc)^n} \qquad (5)$$

where: Vab = maximal feeding rate of protozoa on bacteria (day^{-1})
Ac = amoebal C ($\mu g\ C\ g^{-1}$ soil)
Bc = bacterial C ($\mu g\ C\ g^{-1}$ soil)
Kab = half saturation constant for protozoan consumption of bacteria ($\mu g\ C\ g^{-1}$ soil)
n = nondimensional shape parameter set to 10

Protozoa were assumed to digest only a fraction (Ap) of the bacteria consumed. Thirty-five percent of the unassimilated fraction was released to the soluble C pool and 65% was released to the active C pool (Hunt et al. 1984). Rate of protozoan CO_2-C evolution (Rp) ($\mu g\ C\ g^{-1}$ soil day^{-1}) was calculated similar to bacterial CO_2-C evolution (Hunt et al. 1984):

$$Rp = (Pab \times Ap \times (1-YP)) + (Ac \times Mp) \qquad (6)$$

where: Ap = fraction of bacteria digested by protozoa (unitless)
YP = maximum possible yield of protozoa for utilization of digested bacteria (unitless)
Mp = protozoan maintenance rate constant (day^{-1})

Since most of the amoeba encysted after Day 7, the maintenance rate constant was reduced after this time. No protozoan death was considered in the model since it was assumed that no significant death would occur within the 21 day incubation.

Active C was transferred very slowly to the stable C pool by a first order kinetic reaction that was dependent on the size of the active C pool. This simulated the chemical stabilization of active organic matter to more resistant forms (Juma and Paul 1981).

N Flows between State Variables

Bacterial uptake of NO_3-N and NH_4-N, and mineralization of NH_4-N by bacteria, was determined by the bacterial C/N ratio. The C/N ratio of bacteria was allowed to vary within a set range before N immobilization or mineralization occurred (Hunt et al. 1984). If the consumption of substrate resulted in a bacterial C/N ratio above 4.5 some NO_3-N and NH_4-N would be immobilized to bring the C/N ratio down to 4.5. However, if loss of bacterial C as CO_2-C resulted in a C/N ratio less than 4.25 then NH_4-N would immediately be mineralized to raise the C/N ratio up to 4.25.

Michaelis Menten equations were used to calculate the bacterial uptake (Un) of soluble mineral N (NO_3-N and of NH_4-N) (Hunt et al. 1984):

$$\mathrm{Un} = \frac{V \times C \times Bc}{K + C} \tag{7}$$

where: V = maximal uptake rate of NO_3 or NH_4 (μg N μg^{-1} bacterial C, day^{-1})
C = NO_3 or NH_4 concentration (μg N g^{-1} soil)
K = half saturation constant for NO_3 or NH_4 (μg N g^{-1} soil)

The C/N ratio of protozoa was held constant at 7. If CO_2-C evolution resulted in a C/N ratio less than 7.0 the excess N would immediately be mineralized as NH_4-N to raise the C/N ratio back to 7.0 (Hunt et al. 1984). The flows between other N pools in the N submodel were directly related to the C flows between the corresponding C pools. The N flows were calculated by dividing the C flows by the C/N ratio of the pool from which the flow was originating.

^{14}C and ^{15}N Flows between State Variables

The flow of ^{14}C between pools (Bq g^{-1} soil day^{-1}) was calculated by multiplying the C flow rate (μg C g^{-1} soil day^{-1}) by the specific activity (Bq μg^{-1} C) of the pool from which the ^{14}C was originating. Similarly, the flow of ^{15}N between pools (μg ^{15}N g^{-1} soil day^{-1}) was calculated by multiplying the N flow rate (μg N g^{-1} soil day^{-1}) by the atomic % ^{15}N excess (%) of the pool from which the ^{15}N was originating.

Parameters

The model was first initialized with parameters used by Juma and Paul (1981) and Hunt et al. (1984). However, some parameters were adjusted by iteration to improve the fit of the model to the experimental data from Soil B. Changes in parameters were expected since the model of Juma and Paul (1981) was designed for soil containing a natural suite of organisms, and since tracers provide additional information for model fitting. The parameters used in the model fitted to Soil B are presented in Table VII.2.

Table VII.2. Parameters Used in the Model Fitted to Soil B (clay loam)

Parameter Symbol	Parameter Description	Parameter[a] Value	Origin of Value
Vg	maximal glucose C uptake rate	7.2	Hunt et al. (1984)
Kg	half saturation constant for bacteria uptake of glucose	580	Hunt et al. (1984)
kso	rate constant for bacterial uptake of soluble C	0.5	reduced from Juma and Paul (1981) metabolic C
ka	rate constant for bacterial uptake of active C	0.00095	reduced from Juma and Paul (1981)
kst	rate constant for bacterial uptake of stable C	0.0004	reduced from Juma and Paul (1981) metabolic C
kast	rate constant for transfer of active C to stable C	0.0005	Juma and Paul (1981)
YBg	maximum possible yield of bacterial consumption of glucose	0.525	fitted to data
YBso	maximum possible yield of bacterial consumption of soluble C	0.40	from Juma and Paul (1981) metabolic C
YBa	maximum possible yield of bacterial consumption of active C	0.40	Juma and Paul (1981)
YBst	maximum possible yield of bacterial consumption of stable C	0.40	Juma and Paul (1981)
Mb	bacterial maintenance rate constant	0.0025	fitted to data
Kdb	bacterial death rate constant	0.075	fitted to data
Vab	maximal feeding rate of protozoa on bacteria	3.6	fitted to data
Kab	half saturation for protozoan consumption of bacteria	175	fitted to data

[a] see text for units

Table VII.2. Continued

Parameter Symbol	Parameter Description	Parameter Value	Origin of Value
Ap	fraction of bacteria digested by protozoa	0.60	fitted to data
YP	maximum yield of protozoa for digested bacteria	0.60	Hunt et al. (1984)
Mp	protozoa maintenance rate constant	0.025, 0.0025	Hunt et al. (1984)
Vno3	maximal bacterial NO_3-N uptake rate	6.72	fitted to data
Vnh4	maximal bacterial NH_4-N uptake rate	3.36	fitted to data
Kno3	half saturation constant for bacterial uptake of NO_3-N	100	fitted to data
Knh4	half saturation constant for bacterial uptake of NH_4-N	100	Hunt et al. (1984)

Results

Model Performance for Soil B Data

Generally, the model output fit the data from Soil B very well. The model closely simulated bacterial numbers but underestimated amoebal numbers on Day 12 and 21 (Fig. VII.2). Predicted CO_2-C evolution was underestimated by about 12% during the first four days and overestimated by about 8% on Day 21 (Fig. VII.3). Predicted CO_2-^{14}C recovery fit the data very well, with predicted values falling within the standard error bars of the experimental data (Fig. VII.3).

The model underestimated NO_3-N by 1 to 5 $\mu g\ g^{-1}$ soil (Fig. VII.4). Predicted NH_4-N was greater than the experimental data by about 15% for the protozoa-plus treatment on Day 7 and Day 12, but the model output matched the data for the protozoa-minus treatment (Fig. VII.4). Predicted NO_3-^{15}N recovery

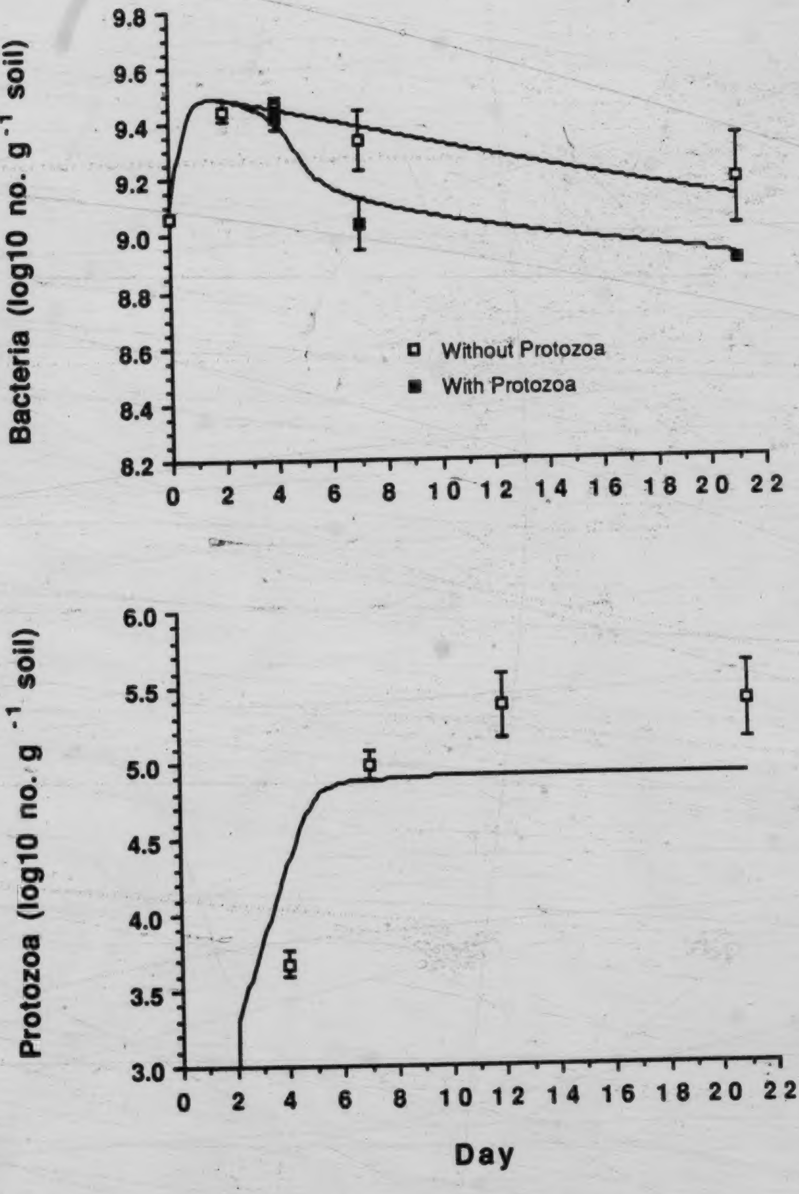

Figure VII.2. Comparison of Model Output (continuous lines) and Experimental Data (symbols and standard error bars) for Bacterial Numbers and Total Protozoa Numbers in Soil B

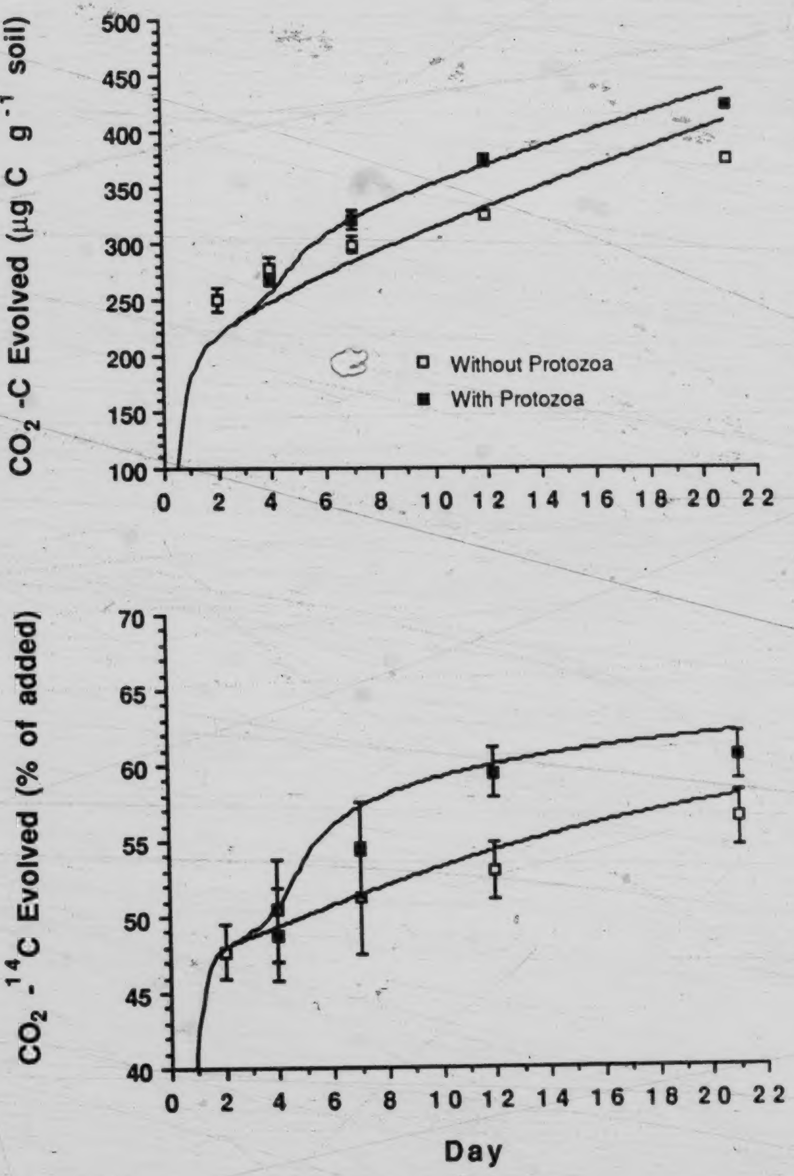

Figure VII.3. Comparison of Model Output (continuous lines) and Experimental Data (symbols and standard error bars) for CO$_2$-C Evolution and CO$_2$-^{14}C Recovery in Soil B

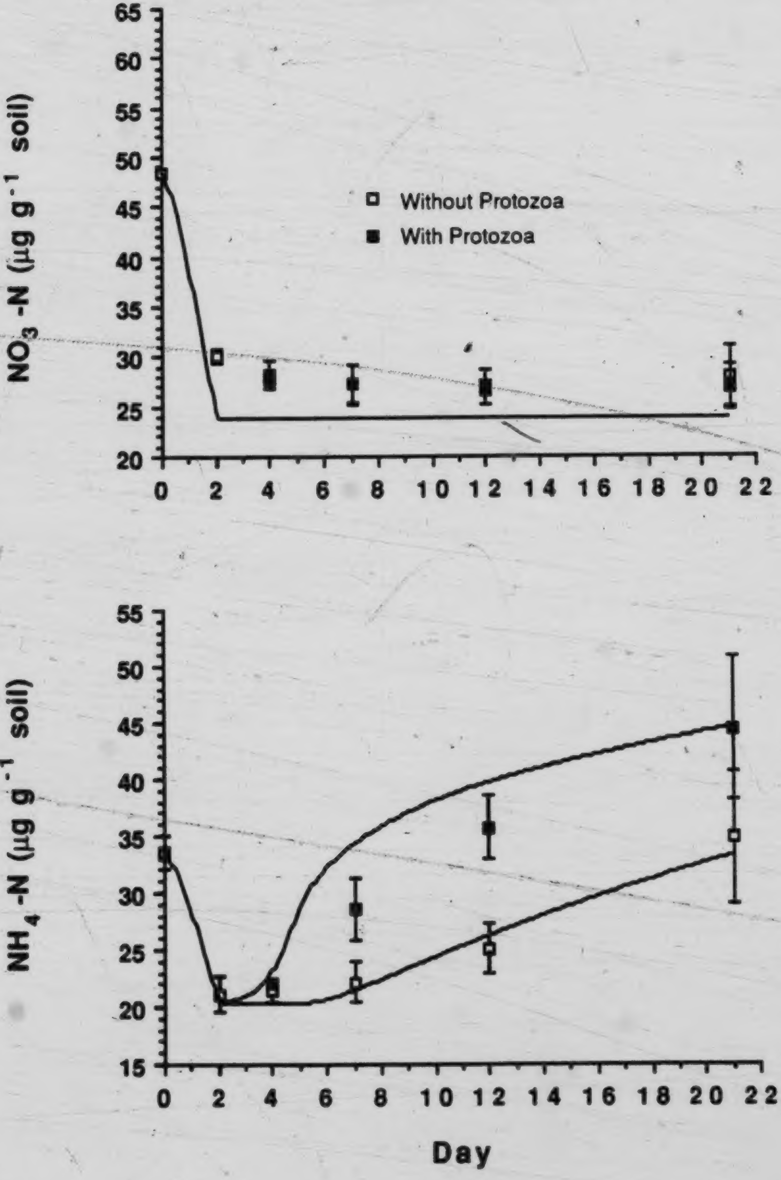

Figure VII.4. Comparison of Model Output (continuous lines) and Experimental Data (symbols and standard error bars) for NH_4-N and NO_3-N Concentration in Soil B

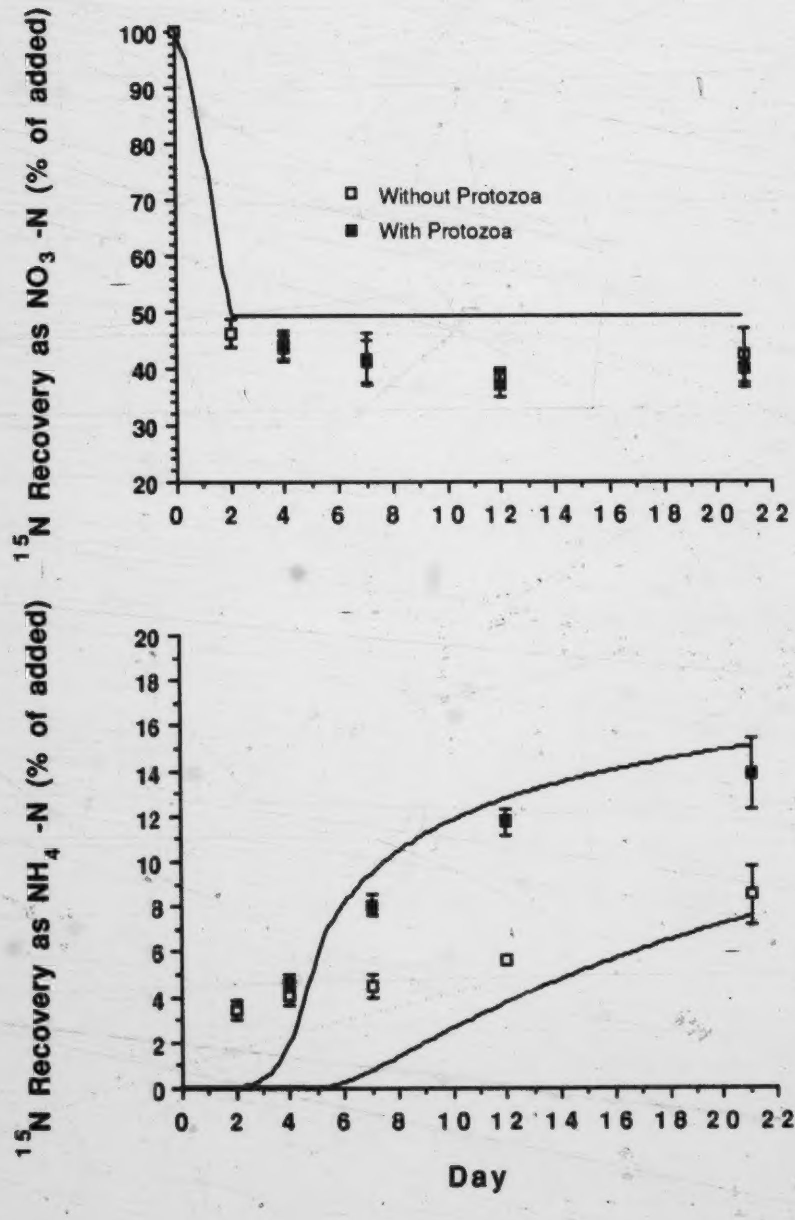

Figure VII.5. Comparison of Model Output (continuous lines) and Experimental Data (symbols and standard error bars) for NH_4-^{15}N and NO_3-^{15}N Recovery in Soil B

Table VII.3. Parameter Changes Made to Adapt the Model to Soil C (sandy loam)

Parameter[a] Symbol	Parameter Value	Justification for Change of Value
YBg	0.50	fitted to data to account for greater CO_2-^{14}C evolution from Soil C
Kab	50	fitted to data to account for greater accessibility of bacteria to protozoa in Soil C
Vno3	8.4	NO_3-N and NH_4-N uptake parameters were increased to account for
Vnh4	6.72	the more rapid mineral N uptake in Soil C
Kno3	80	

[a] see Table VII.2 for description of parameters

and NH_4-^{15}N recovery for the protozoa-plus treatment mimicked the experimental data, however the model underestimated NH_4-^{15}N recovery for the protozoa-plus treatment by about 4% ^{15}N recovery during the first 12 days (Fig. VII.5).

Model Validation

The model was validated on data from Soil C. Initial pool sizes are given in Table VII.1. A minimum of parameter changes were made to account for the coarser texture of Soil C (Table VII.3). Maximum uptake constants of NH_4-N and NO_3-N were increased by 25% and 100%, respectively. The major change to the model was the reduction of the half saturation constant for the protozoan consumption of bacteria (Kab), from 175 to 50. This greatly increased the flow of C from bacteria to protozoa, thereby simulating the lesser protection of bacteria from protozoan predation in the coarse-textured soil.

The model mimicked bacterial and amoebal numbers (Fig. VII.6). Predicted CO_2-C evolution and CO_2-^{14}C recovery were lowered as much as 20%

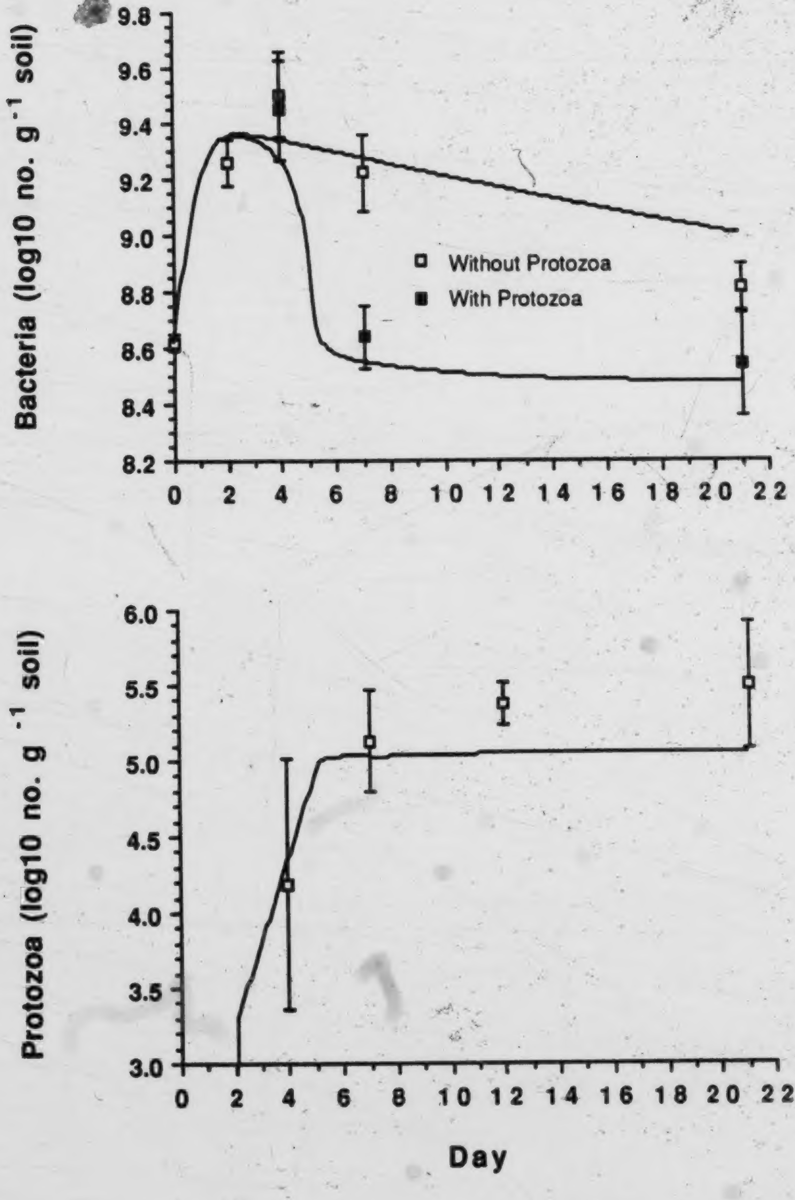

Figure VII.6. Comparison of Model Output (continuous lines) and Experimental Data (symbols and standard error bars) for Bacterial Numbers and Total Protozoa Numbers in Soil C

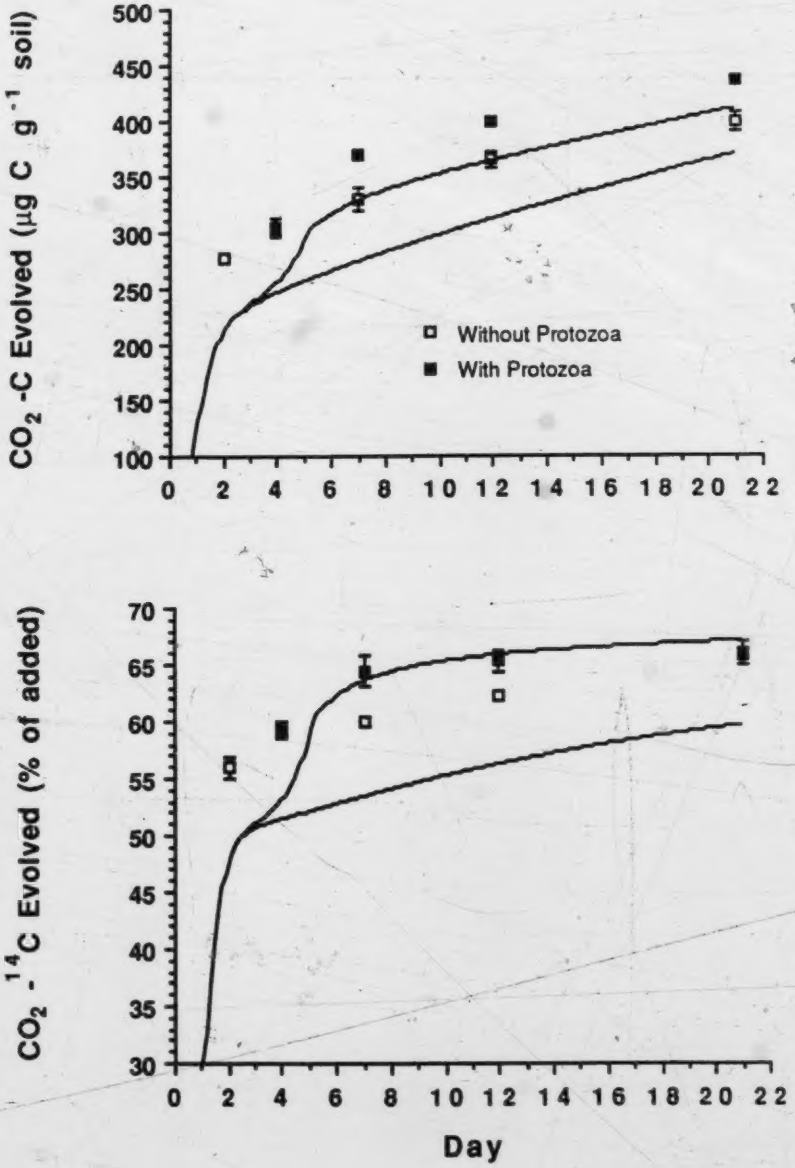

Figure VII.7. Comparison of Model Output (continuous lines) and Experimental Data (symbols and standard error bars) for CO_2-C Evolution and CO_2-^{14}C Recovery in Soil C

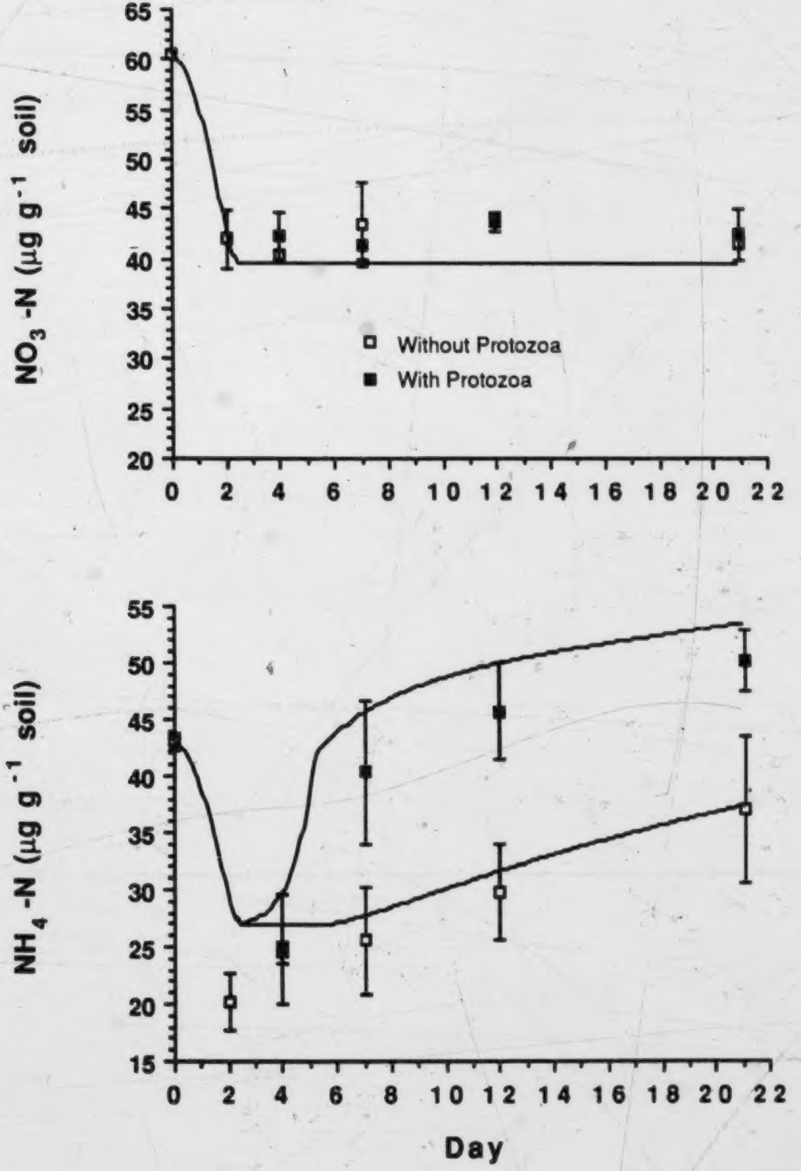

Figure VII.8. Comparison of Model Output (continuous lines) and Experimental Data (symbols and standard error bars) for NO_3-N and NH_4-N Concentration in Soil C

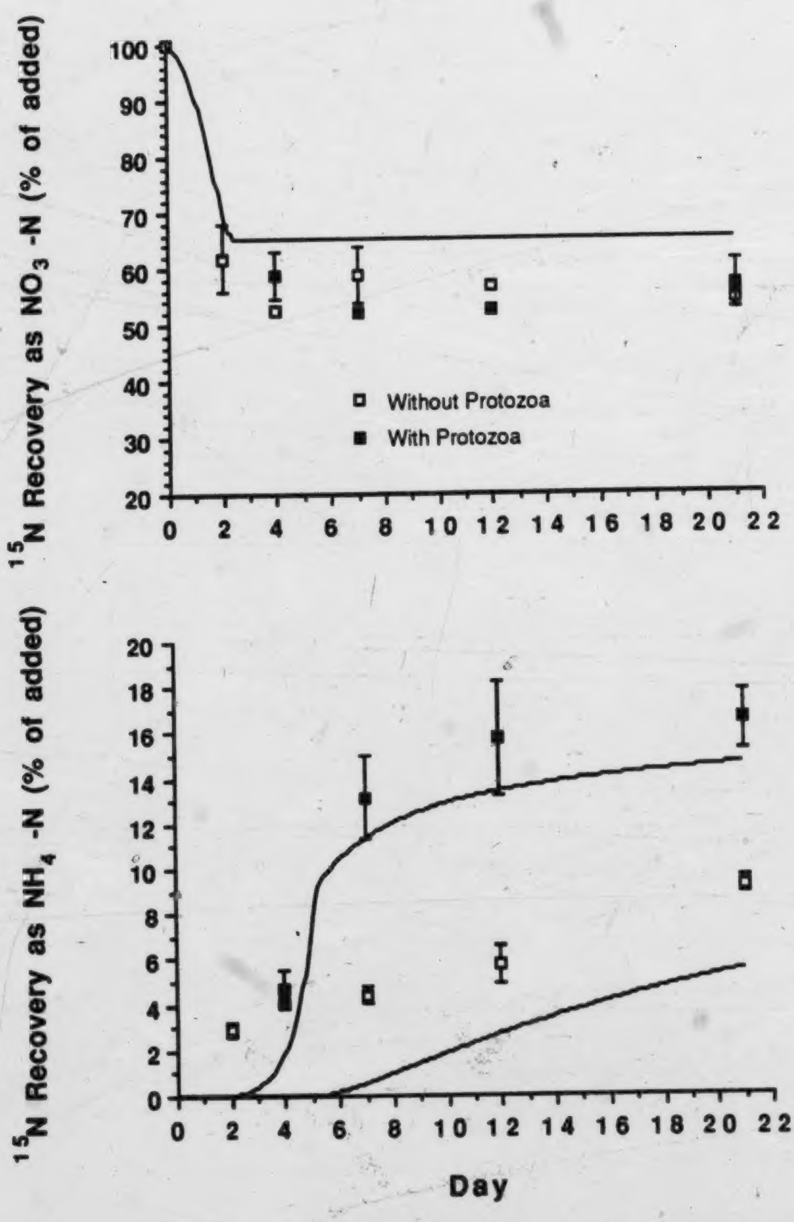

Figure VII.9. Comparison of Model Output (continuous lines) and Experimental Data (symbols and standard error bars) for NO_3-^{15}N and NH_4-^{15}N Recovery in Soil C

and 13%, respectively, from the experimental data (Fig. VII.7). Predicted CO_2-C evolution and

CO_2-^{14}C recovery could have been increased by reducing YBg below 0.5, however this was not done because initial ^{15}N immobilization would be greatly reduced.

Predicted NO_3-N concentration closely matched the experimental data (Fig. VII.8). The model output of NH_4-N concentration fit within the standard deviation bars of most data, although predicted values were greater than the means by as much as 13% (Fig. VII.8). Predicted NO_3-^{15}N recovery was greater than the data by as much as 15% ^{15}N recovery (Fig. VII.9). NH_4-^{15}N recovery was underestimated in both treatments by as much as 4% ^{15}N recovery. Predicted NH_4-^{15}N recovery in the protozoa-plus treatment was usually within, or very close to, the standard deviation bars of the data (Fig. VII.9).

Model Outputs for Two Soils With and Without Protozoa

It is easier to calculate the turnover of soil pools if the system is under steady state conditions (Shipley and Clark 1972). However, a perturbed soil system is rarely under steady state conditions so an alternative way to approximate turnover is to consider total inputs and outputs of specific pools over a certain time interval. The incubation period was divided into a period of rapid changes (Day 0-12), and a period of slower changes (Day 12-21). The total pool inputs and outputs, along with initial and final pool sizes for the two time intervals were determined for the two soils, with and without protozoa (Tables VII.4 to VII.7).

Protozoa-Minus Treatments

The CO_2-C evolved from Soil B (331 μg C g^{-1} soil) during the first 12 days was mainly due to glucose addition (360 x 0.475 = 171 μg C g^{-1} soil) and cycling of C in the soil (160 µg C g^{-1} soil). During this interval, inputs into bacteria were 5.5-fold greater than the inital pool size. This was produced by a rapid uptake of glucose C (360 µg C g^{-1} soil) and C from other soil pools. Bacterial outputs were mainly respiration (331 µg C g^{-1} soil), and death (542 - 331 = 212 µg C g^{-1} soil) which was partitioned into soluble (74 µg C g^{-1} soil) and active organic matter (138 µg C g^{-1} soil). A small portion of active organic matter (8 µg C g^{-1} soil) was transferred into the stable pool. Bacterial and active C pools increased but the soluble and stable C pools decreased over the 12 days. The ^{14}C was mainly partitioned into CO_2-C (54%), bacteria (24%) and active organic matter (20%) by Day 12. The death of bacteria contributed to the soluble (509 Bq g^{-1} soil) and active (944 Bq g^{-1} soil) ^{14}C pools. The difference between total bacterial ^{14}C inputs (5126 Bq g^{-1} soil) and added glucose ^{14}C glucose (4680 Bq g^{-1} soil) represented the internal cycling of added C.

Addition of glucose to Soil B caused immobilization of NO_3-N (25 µg N g^{-1} soil) and NH_4-N (13 µg N g^{-1} soil) within the first two days. Bacterial mineralization was 6 µg N g^{-1} soil and bacterial death of 48 µg N g^{-1} soil was partitioned into soluble (17 µg N g^{-1} soil) and active N (31 µg N g^{-1} soil). Bacterial N and active N increased during the first interval, however soluble and stable N showed a net decrease. The NO_3-^{15}N immobilized (698 ng ^{15}N g^{-1} soil) was mainly partitioned into bacteria (51%) active (29%) and stable (10%) OM by Day 12. Bacterial mineralization was 51 ng ^{15}N g^{-1} soil and bacterial death of 415 ng ^{15}N g^{-1} soil was partitioned into soluble N (145 ng ^{15}N g^{-1} soil) and active N (270 ng ^{15}N g^{-1} soil). The difference between total bacterial ^{15}N inputs (824 ng ^{15}N g^{-1} soil) and ^{15}N immoblized (698 ng ^{15}N g^{-1} soil) represented the internal cycling of added N.

Table VII.4. Initial Pool Size and Simulated Inputs and Outputs Between Day 0 and Day 12 and Between Day 12 and Day 21 for Soil B Protozoa-Minus Treatment

Variable	Day 0 Pool Size	Inputs	Outputs	Day 12 Pool Size	Inputs	Outputs	Day 21 Pool Size
			Carbon (μg g^{-1} soil)				
Bacteria	111	614	542	183	123	180	126
Soluble	80	74	143	11	36	39	7
Active	1308	138	24	1422	67	19	1470
Stable	19855	8	96	19768	7	70	19704
CO_2-C	0	331	0	331	77	0	408
			Carbon-^{14}C (Bq g^{-1} soil)				
Bacteria	0	5126	3992	1134	243	744	632
Soluble	0	509	440	68	203	233	38
Active	0	944	9	936	377	15	1297
Stable	0	3	0.005	3	5	0.02	8
CO_2-C	0	2539	0	2539	165	0	2704
			Nitrogen (μg g^{-1} soil)				
Bacteria	26	71	54	44	18	32	30
Soluble	8	17	22	3	9	9	2
Active	218	31	4	245	16	3	258
Stable	1805	1	9	1798	1	6	1792
NO_3-N	48	0	25	24	0	0	24
NH_4-N	34	6	13	26	7	0	33
			Nitrogen-^{15}N (ng g^{-1} soil)				
Bacteria	0	824	466	358	76	235	199
Soluble	0	145	124	21	64	73	12
Active	0	270	69	201	119	100	220
Stable	0	67	0.11	67	98	0.4	164
NO_3-N	1370	0	698	672	0	0	672
NH_4-N	0	51	0	51	52	0	103

Table VII.5. Initial Pool Size and Simulated Inputs and Outputs Between Day 0 and Day 12 and Between Day 12 and Day 21 for Soil C Protozoa-Minus Treatment

Variable	Day 0 Pool Size	Inputs	Outputs	Day 12 Pool Size	Inputs	Outputs	Day 21 Pool Size
Carbon (µg g^{-1} soil)							
Bacteria	40	568	467	142	92	137	97
Soluble	80	55	126	8	28	31	6
Active	1638	102	29	1710	51	23	1739
Stable	13134	10	63	13081	8	47	13042
CO_2-C	0	310	0	310	58	0	368
Carbon-^{14}C (Bq g^{-1} soil)							
Bacteria	0	5090	3978	1112	237	729	620
Soluble	0	472	405	67	199	228	38
Active	0	876	8	868	369	14	1223
Stable	0	3	0.004	3	5	0.02	7
CO_2-C	0	2631	0	2631	161	0	2792
Nitrogen (µg g^{-1} soil)							
Bacteria	9	65	40	34	14	25	23
Soluble	8	12	18	2	6	7	1
Active	273	23	5	291	12	4	300
Stable	1194	2	6	1190	1	4	1187
NO_3-N	61	0	21	39	0	0	39
NH_4-N	43	5	16	32	5	0	37
Nitrogen-^{15}N (ng g^{-1} soil)							
Bacteria	0	563	316	247	52	164	135
Soluble	0	98	83	15	44	51	8
Active	0	181	45	136	82	68	150
Stable	0	44	0.07	44	66	0.3	110
NO_3-N	1370	0	479	891	0	0	891
NH_4-N	0	37	0	37	38	0	75

Bacterial C in Soil B decreased between Day 12 and 21 because they were mainly being sustained by C from the soluble C (39 μg g^{-1} soil), active C (19 μg g^{-1} soil) and stable C (70 μg g^{-1} soil). Although the rate constant of stable C was 25% of active C, the amount of stable C taken up was almost 4-fold greater because the stable C pool was 15 times greater. Respiration and all C pools except active C decreased during this interval. Bacterial mineralization was 7 μg N g^{-1} soil while immobilization was 0 μg N g^{-1} soil because the system was C limited. At the end of 21 days, 28% of added ^{14}C and 31% of immobilized ^{15}N were recovered in active pools. Recovery of recently added C and N increased in the slowly cycling active and stable pools.

The main differences between Soil B and Soil C protozoa-minus treatments were the smaller initial bacterial and stable C and N pools sizes in Soil C, the larger active pool in Soil C, and the greater loss of glucose-^{14}C as CO_2-^{14}C in Soil C which resulted in less immobilization of NO_3-^{15}N into organic N pools (Table VII.4 and VII.5). The trends in changes of pool size changes, and inputs and outputs were otherwise similar in both soils.

Effect of Protozoa Addition on Model Outputs for Soil B

Most of the effects of protozoa on C, N, ^{14}C and ^{15}N dynamics were common to both soils. For simplicity, this section will describe the effects of protozoa for Soil B only. The next section will describe how textural differences between the two soils impacted on the protozoa effects on C, N, ^{14}C and ^{15}N dynamics.

Protozoa increased the output of bacterial C by 16%, compared to the protozoa-minus treatment, between Day 0 and Day 12, and reduced bacterial C inputs and outputs in the later interval (Table VII.4 and VII.6). Inputs and outputs of soluble C, active C and stable C were not greatly affected by protozoa during the early interval period. However, protozoa greatly reduced inputs into the active C pool during the later interval, while outputs remained the same, resulting in a decrease in the size of the active C pool on Day 21. Inputs and outputs of soluble C were reduced in the protozoa-plus treatment between Day 12 and 21. Protozoa directly increased total CO_2-C evolution by 11 % in the early interval. CO_2-C evolution during the later interval was less in the protozoa-plus treatment, compared to the protozoa-minus treatment, however total evolution for the entire 21 day period was greatest in the protozoa-plus treatment.

Protozoa increased the output of bacterial N by 37% between Day 0 and Day 12, and reduced bacterial N inputs and outputs in the later interval. Inputs and outputs of soluble N, active N and stable N were not greatly affected by protozoa during the early interval. Inputs and outputs of soluble N were reduced by protozoa in the later interval but the change in Day 21 pool size was only slight. Active N outputs were reduced by 38% in the later interval. Protozoa had no effect on the immobilization of NO_3-N. Protozoa mineralized twice as much NH_4-N as did bacteria during the early interval, thereby increasing total NH_4-N three-fold compared to the protozoa-minus treatment. Protozoa had a much lesser effect of increasing NH_4-N mineralization during the later interval.

Addition of glucose-^{14}C and KNO_3-^{15}N allowed the bacteria to be labelled so the fate of bacterial C and N could be traced independently of C and N originating from other soil C and N pools. Protozoa increased the output of ^{14}C from the bacterial pool during the first 12 days so the bacterial ^{14}C in the protozoa-plus treatment was half the size of bacteria in the protozoa-minus treatment on Day 12. Bacterial ^{14}C inputs and outputs were less in the protozoa-plus treatment than in the protozoa-minus treatment during the later interval. Soluble ^{14}C outputs in the protozoa-plus treatment were increased in the early interval and both inputs and outputs decreased in the later interval, compared to the protozoa-minus treatment. Pool

Table VII.6. Initial Pool Size and Simulated Inputs and Outputs Between Day 0 and Day 12 and Between Day 12 and Day 21 for Soil B Protozoa-Plus Treatment

Variable	Day 0 Pool Size	Inputs	Outputs	Day 12 Pool Size	Inputs	Outputs	Day 21 Pool Size
Carbon (μg g^{-1} soil)							
Bacteria	111	618	627	101	107	129	80
Amoeba	1.1[a]	142	93	48	2	3	48
Soluble	80	74	148	7	21	24	4
Active	1308	138	24	1422	40	19	1443
Stable	19855	8	95	19768	6	71	19704
Tot CO_2-C	0	368	0	368	68	0	436
Bac CO_2-C	0	331	0	331	66	0	397
Am CO_2-C	0	37	0	37	2	0	39
Carbon-^{14}C (Bq g^{-1} soil)							
Bacteria	0	5153	4587	567	134	389	312
Amoeba	0	985	655	330	12	16	325
Soluble	0	509	467	42	103	125	19
Active	0	945	10	935	191	13	1112
Stable	0	3	0.006	3	5	0.02	8
Tot CO2-C	0	2803	0	2803	100	0	2903
Bac CO_2-C	0	2542	0	2542	90	0	2632
Am CO_2-C	0	261	0	261	10	0	271
Nitrogen (μg g^{-1} soil)							
Bacteria	26	72	74	24	14	20	19
Amoeba	0.2[a]	33	26	7	0.6	0.6	7
Soluble	8	17	23	2	5	6	1
Active	218	31	4	245	10	3	251
Stable	1805	1	7	1798	1	6	1792
NO_3-N	48	0	25	24	0	0	24
Tot NH_4-N	34	19	13	40	4.9	0	45
Bac NH_4-N	-	6	13	-	4.6	0	-
Am NH_4-N	-	13	0	-	0.4	0	-
Nitrogen-^{15}N (ng g^{-1} soil)							
Bacteria	0	833	652	181	42	124	99
Amoeba	0	307	246	60	3	5	59
Soluble	0	146	133	13	33	40	6
Active	0	271	79	193	61	84	169
Stable	0	77	0.1	77	83	0.4	159
NO_3-N	1370	0	698	672	0	0	672
Tot NH_4-N	0	174	0	174	32	0	206
Bac NH_4-N	0	52	0	52	29	0	80
Am NH_4-N	0	123	0	123	3	0	126

[a] initial size on Day 2 inoculation

Table VII.7. Initial Pool Size and Simulated Inputs and Outputs Between Day 0 and Day 12 and Between Day 12 and Day 21 for Soil C Protozoa-Plus Treatment

Variable	Day 0 Pool Size	Inputs	Outputs	Day 12 Pool Size	Inputs	Outputs	Day 21 Pool Size
Carbon (μg g^{-1} soil)							
Bacteria	40	574	584	30	71	73	29
Amoeba	1.1[a]	194	128	66	10	8	68
Soluble	80	55	132	3	8	10	2
Active	1638	102	29	1711	15	22	1704
Stable	13134	10	63	13081	7	47	13041
Tot CO_2-C	0	362	0	362	47	0	409
Bac CO_2-C	0	311	0	311	43	0	354
Am CO_2-C	0	51	0	51	4	0	55
Carbon-^{14}C (Bq g^{-1} soil)							
Bacteria	0	5138	4987	151	58	142	67
Amoeba	0	1679	1128	551	35	52	534
Soluble	0	474	452	22	35	51	7
Active	0	880	10	870	66	12	924
Stable	0	3	0.006	3	4	0.02	7
Tot CO2-C	0	2974	0	3082	58	0	3140
Bac CO_2-C	0	2651	0	2637	37	0	2674
Am CO_2-C	0	445	0	445	21	0	465
Nitrogen (μg g^{-1} soil)							
Bacteria	9	66	68	7	9	10	7
Amoeba	0.2[a]	46	37	9	2	1	10
Soluble	8	13	20	1	2	2	0.5
Active	273	23	5	291	4	4	291
Stable	1194	2	6	1190	1	4	1187
NO_3-N	61	0	21	39	0	0	39
Tot NH_4-N	43	23	16	50	3.6	0	53
Bac NH_4-N	-	5	16	-	2.4	0	-
Am NH_4-N	-	18	0	-	1.1	0	-
Nitrogen-^{15}N (ng g^{-1} soil)							
Bacteria	0	574	538	37	13	34	16
Amoeba	0	375	305	70	8	15	64
Soluble	0	100	95	5	8	12	1
Active	0	185	58	127	15	50	92
Stable	0	57	0.1	57	49	0.3	106
NO_3-N	1370	-	479	891	0	0	891
Tot NH_4-N	0	183	0.02	183	17	0	200
Bac NH_4-N	0	31	0.02	31	9	0	39
Am NH_4-N	0	153	0	153	8	0	161

[a] initial size on Day 2 inoculation

sizes were smaller in the protozoa-plus treatment on Day 12 and 21. Active ^{14}C outputs were not significantly affected by the presence of protozoa, however inputs were greatly reduced during the later period, resulting in a smaller active ^{14}C pool size on Day 21 in the protozoa-plus treatment, compared to the protozoa-minus treatment. The stable ^{14}C pool was very small and protozoa had little effect on inputs and outputs. The presence of protozoa increased total CO_2-^{14}C evolution in the early interval by 10% over the protozoa-minus treatment. A small portion of this increase was due to increased bacterial respiration, but 99% of the total increase was from protozoan respiration of bacterial biomass. CO_2-^{14}C evolution after Day 12 was less in the protozoa-plus treatment, compared to the protozoa-minus treatment.

Protozoa increased the output of bacterial ^{15}N so much that the pool size on Day 12 was 50% of the pool size in the protozoa-minus treatment. Inputs and outputs were less than in the protozoa-minus treatment after Day 12. Output of soluble ^{15}N and active ^{15}N were increased by protozoa during the first 12 days, but both inputs and outputs were decreased by protozoa after Day 12. The presence of protozoa reduced the final pool sizes of bacterial ^{15}N, soluble ^{15}N and active ^{15}N. Stable ^{15}N was reduced very slightly by the presence of protozoa. The presence of protozoa had no effect on NO_3-^{15}N changes but total net NH_4-^{15}N mineralization was increased 3.4-fold by Day 12 and 2-fold by Day 21 when protozoa were present. All of this increase was due to the mineralization of NH_4-^{15}N by protozoa.

Effect of Texture on Bacterial - Protozoan Interactions and C and N Dynamics

Protozoa had greater access to bacterial biomass in Soil C than in Soil B, consequently outputs of bacterial-C, -N, -^{14}C and -^{15}N were proportionally greater in the coarse-textured soil (Table VII.8). As a result of greater predation of bacteria, protozoa in Soil C increased total CO_2-C evolution, CO_2-^{14}C recovery,

Table VII.8. Percent Increase or Decrease in Pool Sizes and Mineralization in the Protozoa-Plus Treatment as Compared to the Protozoa-Minus Treatment

	Day 12		Day 21	
Variable	Soil B	Soil C	Soil B	Soil C
Bacteria C	-45	-79	-37	-70
Soluble C	-35	-61	-38	-67
Active C	+0.01	+0.003	-2	-2
Stable C	0	0	0	0
Total CO_2-C	+11	+17	+7	+11
Bacteria N	-44	-78	-37	-70
Soluble N	-34	-61	-38	-67
Active N	+0,04	+0.08	-2	-3
Stable N	0	0	0	0
Total NH_4-N Mineralization	+230	+379	+88	+152
Bacteria ^{14}C	-50	-86	-51	-89
Soluble ^{14}C	-38	-67	-50	-82
Active ^{14}C	-0.05	+0.3	-14	-24
Stable ^{14}C	+13	+25	-0.09	-0.7
Total CO_2-^{14}C	+10	+17	+7	+12
Bacteria ^{15}N	-49	-85	-50	-88
Soluble ^{15}N	-38	-65	-50	-88
Active ^{15}N	-4	-7	-23	-21
Stable ^{15}N	+15	+28	+3	+4
Total NH_4-^{15}N Mineralization	+243	+402	+102	+167

NH_4-N mineralization and NH_4-^{15}N mineralization more than protozoa in Soil B (Table VII.8). Texture had an effect on the relative pool sizes of bacteria and protozoa (Table VII.6 and VII.7). In Soil B the pool sizes of bacterial C, N and ^{15}N on Day 21 were greater than the corresponding amoebal pools. However, in Soil C all protozoan pools were greater than the corresponding bacterial pools on Day 21.

Discussion

Simulation modelling provided a test of existing information on soil organic matter dynamics and bacteria-protozoa interactions, and it allowed for rigorous testing of the hypotheses put forward in Chapter 5 and 6. Thus, a quantitative link was made between microbial-faunal populations and C and N cycling in soil. Model development and fitting to the experimental data provided insight into the variables and parameters used in the model, while comparison of Soil B and Soil C model outputs provided insight into the impact of soil texture on bacterial-protozoan interactions and C and N dynamics.

Model Development and Fitting

During model development it was necessary to make several changes from the models of Hunt et al. (1984) and Juma and Paul (1981) in order to allow simulated outputs to match the experimental data. The yield efficiency of glucose (YBg) was reduced from 0.70 (Hunt et al. 1984) to 0.525 to account for the initial CO_2-^{14}C evolution from Soil B. This parameter was further reduced to 0.50 in Soil C, yet initial CO_2-^{14}C evolution was still underestimated. Further reduction of YBg in Soil C would greatly affect N transfers unless the C/N ratio of bacteria in Soil C was lowered to increase initial immobilization of NH_4-N and NO_3-N. The C yield efficiencies used for Soil B and Soil C are within the range presented in the literature (McGill et al. 1981). Van Veen and Kuikman (1990) state that production efficiencies of soil organisms may be greater in fine- versus coarse-textured soils.

The rate constants for the bacterial uptake of soluble C (kso), active C (ka), and stable C (kst) were all reduced from values used by Juma and Paul (1981) to prevent excessive CO_2-C and CO_2-^{14}C evolution during the later two-thirds of the incubation. This was done because a single *Pseudomonas* species was not expected to decompose soil organic matter as effectively as an assemblage of numerous bacterial and fungal species found in natural soil.

Unlike the model of Hunt et al. (1984) the present model was designed to account for bacterial death, which was more prevalent in the present study. The rate constant (Kdb) was determined iteratively with consideration of the measureable decline in cell numbers. Cell death was considered independently from predation-induced death unlike some other models (McGill et al. 1981). The maintenance rate constant (Mb) was much lower than that used by Hunt et al. (1984) since dying cells were assumed to have much lower energy requirements than stable or growing populations.

The present model used the same bacterial NH_4-N uptake equation as Hunt et al. (1984), however the maximal uptake rate had to be increased to account for the rapid immobilization which was observed during the first three days of the experiment. Unlike the models of Hunt et al. (1984) and Juma and Paul (1981), the present model considered simultaneous uptake of NO_3-N and NH_4-N. Microorganisms preferentially use NH_4-N over NO_3-N (Paul and Juma 1981), however bacteria rapidly took up more NO_3-N because it was present in higher concentrations than NH_4-N in the present study.

The maximal protozoan predation rate on bacteria (Vab) was increased from the value of 0.3 h^{-1} used by Hunt et al. (1984) to account for the observed rapid decline of bacterial numbers and rapid increases of amoebal numbers, CO_2-C evolution and NH_4-N mineralization. The *Acanthamoeba* used in the present study may have been a smaller but more rapidly growing species than the one used by Hunt et al. (1984). Perhaps soil conditions were more favorable for the growth of the amoeba.

Hunt et al. (1984) found that the amoeba used in their study digested 95% of the bacteria consumed. The same parameter value lead to a significant over estimation of CO_2-C evolution and NH_4-N mineralization in the present model. A value of 60% was chosen in the present model, because it resulted in a closer match of model output to the experimental data. The model of Hunt et al. (1984) did not consider protozoan encystment. During the present laboratory experiment, a large proportion of amoeba became encysted by Day 7 (Fig. V.3). The present model considered encystment by reducing the protozoan maintenance rate constant (Mb) ten-fold after Day 7. There was very little difference in C and N dynamics, compared to a model version which did not consider encystment, since protozoa influence C and N mineralization mainly during growth. However, most protozoan growth occurred during the first seven days of the incubation (Fig. V.3).

Effect of Protozoa on Model Output

Protozoa rapidly reduced the size of the bacterial C and N pools and partioned these elements between protozoan biomass, soluble and active organic matter, and increased CO_2-C evolution and NH_4-N mineralization. Inputs into soil organic C and N pools were not greatly affected during the early interval by the presence of protozoa. Bacterial death was the major input into these pools and bacterial numbers were high for the the first five days in both treatments, therefore death inputs were high too. When protozoa reduced bacterial numbers, and hence reduced bacterial death inputs, the protozoan inputs into the active C and N pools partially compensated for the reduced bacterial death inputs. Inputs into the stable organic matter pools were determined by the size of the active C and N organic matter pools, hence they were quite insensitive to the effect that protozoa had on C and N transfers. Outputs of soluble and active C and N were dependent on pool size, therefore they were relatively unaffected during the 0-12 day interval because inputs were only slightly affected.

Protozoa reduced inputs into soluble and active C and N pools after Day 12 because bacterial biomass was substantially reduced during the early interval, resulting in less bacterial death inputs as well. Also, protozoan inputs into these pools were greatly reduced in the later interval since these inputs occurred during protozoan growth, which essentially ceased during the early interval.

The additional sensitivity of the ^{14}C tracer showed that protozoan predation did slightly increase bacterial C inputs during the early interval. The increased bacterial inputs were responsible for a very small increase in bacterial CO_2-^{14}C evolution when protozoa were present. However, by far the greatest increase in CO_2-^{14}C evolution was directly due to protozoan respiration. Since protozoa were directly responsible for most of the increased NH_4-^{15}N mineralization in the protozoa-plus treatments over the 21 days, protozoa cannot be said to stimulate turnover and increased net N mineralization from bacteria by the excretion of protozoan waste products. Under conditions of N limitation, protozoa may stimulate microbial turnover and decomposition of substrates by partially relieving the N limitation (Robinson et al. 1989). These conditions did not exist in the present study since NO_3-N and NH_4-N were plentiful.

The Effect of Texture on Bacterial-Protozoan Interactions and C and N Dynamics

Reduction of the half saturation constant for the protozoan consumption of bacteria (Kab) effectively adapted the model from a soil in which bacteria were well protected from protozoan predation (Soil B), to a soil in which they were not well protected (Soil C). This change was analagous to changing the shape parameter (n) to alter the protection of bacteria from nematode predation (Hunt et al. 1984). The change made to Kab to adapt the model to Soil C was made iteratively. Future research should be conducted to see if a theoretical or empirical relationship can be made to link soil texture and structure to Kab.

The model confirms the hypothesis put forward in Chapter 6 that protozoa in coarse-textured soil are more effective grazers of bacteria and mineralize more C as CO_2-C and N as NH_4-N than protozoa in fine-textured soil. The model also provided much additional information on the flows of C and N within various soil pools which could not be determined empirically. For example, in fine-textured soil the largest ^{15}N pool on Day 21 was the active pool, followed by the stable, bacterial, amoebal and soluble pools. However, in coarse-textured soil the largest ^{15}N pool was the stable pool, followed by the active, amoebal, bacterial and soluble pools. Thus, soil texture can have a significant effect on bacterial-protozoan interactions and on the dynamics and partitioning of added N.

The model also predicted that mineralization of C and N is rapid when bacterial numbers are increased due to a perturbation. This may have a significant influence in nature where conditions such as wetting and drying and C additions are common.

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Chapter 8. General Discussion and Conclusions

The overall objective of this thesis was to link dynamics of soil organisms with N cycling in some Canadian soils. In order to accomplish this, a project was designed which consisted of a field experiment (Chapters 2 to 4), a laboratory experiment (Chapters 5 and 6) and a simulation model (Chapter 7).

The objective of Chapter 2 was to compare microbial C and the populations of soil faunal organisms in two genetically different soils which were cropped to barley. Microbial C (g m^{-2}), nematode, acari and collembola populations (no. m^{-2}) were greater in the Black Chernozemic soil at Ellerslie than in the Gray Luvisolic soil at Breton. Ten-day laboratory incubation of 0-10 cm soil samples from Ellerslie evolved more CO_2-C (μg C g^{-1} soil) compared to samples from Breton. These results were expected because the Black Chernozemic soil had 2 to 3 times more soil organic C than the Gray Luvisolic soil at Breton. In order to evaluate whether the soil organic C in the two soils was equally available to the biological communities, microbial C, faunal populations and CO_2-C evolution were expressed relative to the amount of soil organic C present in the two soils. Microbial C and protozoa numbers expressed per unit of soil C were significantly greater in the Gray Luvisol, and acari and collembola populations were not significantly different. CO_2-C evolution (mg C g^{-1} C soil) was 2.3-fold greater from the Gray Luvisol than from the Black Chernozem. Although the Gray Luvisol had less organic C than the Black Chernozem, a larger proportion of the organic C in the Gray Luvisol was kinetically active and available to the biological community.

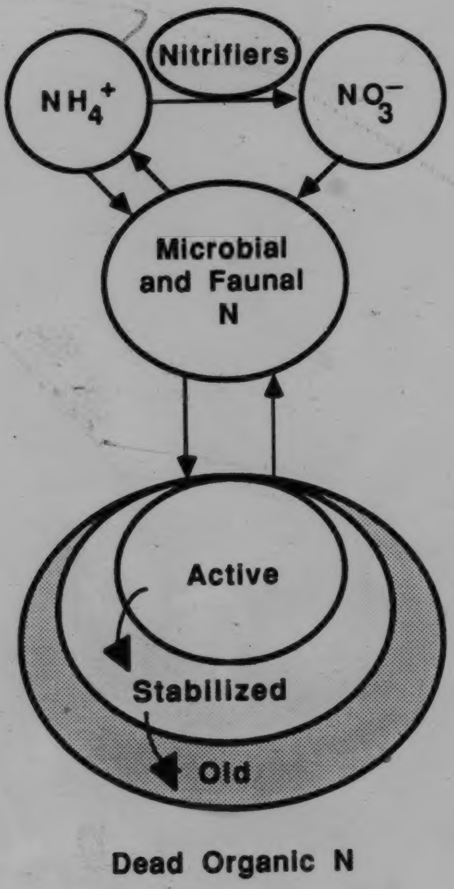
Nitrifiers
NH_4^+
NO_3^-
Microbial and Faunal N
Active
Stabilized
Old
Dead Organic N

Figure VIII.1 Submodel of Soil N Cycle

The objective of Chapter 3 was to compare N dynamics in the same two soils cropped to barley. This research was conducted simultaneously with the research in Chapter 2 with the aim of establishing linkages between soil organisms and N cycling (Fig. VIII.1). Total soil N (g m^{-2}) was 2-3 times greater in the Black Chernozem than in the Gray Luvisol. The two soils showed differences in the relative sizes of kinetically active pools and in relative mineralization rates. Microbial N (0-10 cm) was not significantly different at Ellerslie than at Breton but microbial N made up a larger proportion of total soil N at Breton than at Ellerslie. In the 0-10 cm interval, microbial N was 1.7-fold greater and non-microbial active N was 3-fold greater at Breton compared to Ellerslie when expressed as a proportion of total soil N. Net N mineralization in a 10-day laboratory incubation was 1.4-fold greater in the Black Chernozem (0-10 cm interval) from Ellerslie, compared to the Gray Luvisol from Breton, when expressed per gram of soil. However, net N mineralization in the soil from Breton was double that of the soil from Ellerslie when expressed as a proportion of soil N. Thus, analogous to soil organic C, a larger proportion of the soil organic N in the Gray Luvisol was kinetically active and more available to the biological community than the soil organic N in the Black Chernozem. The proportionally large size of the active pools in the Luvisol and the greater activity of microbial biomass resulted in a greater N mineralization expressed as a proportion of total N in it compare to the Black Chernozem. A larger proportion of N in the Black Chernozem was contained within the stable N and/or the old N fractions .

The reason for the more rapid cycling of the soil organic C and N in the Gray Luvisol than in the Black Chernozem was not known. Differences in soil genesis may have lead to different chemical compositions of the soil organic matter in the two soils (Stewart 1987). Campbell and Souster (1982) found that potentially mineralizable N accounted for a larger proportion of soil N in cultivated Gray Luvisols compared to Black Chernozems. Another possible explanation was related to the different texture and mineralogy in the two soils. The Ap horizon of the Black Chernozem had a finer texture with a higher smectite content than the Ap horizon of the Gray Luvisol (Pawluk 1980, 1986). Smectite clays may stabilize organic matter in soil (Pawluk 1986), thereby reducing its availability to the biota. In addition, van Veen and Kuikman (1990) stated that turnover of the microbial biomass C and N is slower in fine-textured soils and this may be the result of microbial protection against predation and/or the amelioration of harsh environmental conditions.

The work conducted in Chapters 5 and 6 followed from the ideas presented in Chapters 2 and 3 that texture may have played a role in determining the turnover rate of soil C and N by influencing microbial protection. Since a larger proportion of the porosity in fine-textured soils consists of very small pores, compared to the porosity in coarse-textured soils (Papendick and Campbell 1981), it was hypothesized that bacteria in fine-textured soils would be more protected from protozoan predation than bacteria in coarse-textured soils. Since protozoan predation of bacteria results in increased net N mineralization (Coleman et al. 1984) it was also hypothesized that protozoa in coarse-textured soils would be more effective at increasing the mineralization of bacterial N than in fine-textured soils. Pore size distribution is also affected by soil structure, however if soils are of similar genesis and have similar management and cropping histories, then texture will strongly influence soil structure and hence pore size distribution. Three sieved Orthic Black Chernozemic soils of similar management history but different texture (silty clay, clay loam, sandy loam) were used in a microcosm experiment to test the above hypotheses. It was found that an *Acanthamoeba* species more effectively reduced the numbers of a fluorescent *Pseudomonas* species and increased the mineralization of bacterial N in the sandy loam soil than in the two fine-textured soils. Thus, texture influenced the turnover of the microbial biomass and also soil C and soil N, by affecting the protection of the microbial biomass from faunal predation. It was also concluded that protozoa-induced mineralization of bacterial N may be very brief in soil, however this does not diminish the importance of protozoa in the process of N mineralization since microbial activity often occurs in bursts following perturbations, such as aggregate disruption, energy addition or water addition. During these bursts of microbial activity protozoa may make N available to plants which otherwise would be tied up within the bacterial biomass.

Simulation modelling is a means of rigorously testing hypotheses and of integrating existing information. The construction of models helps to identify gaps in knowledge and thus helps to direct the progression of science. In Chapter 7 a simulation model was constructed to simulate the predation of bacteria by protozoa and the resulting flows of C and N obtained in the microcosm experiment (Chapter 5 and 6). The model integrated the concepts of soil organic matter cycling as described in the TRAMIN model (Juma and Paul 1981) and the bacterial-protozoan component of the food web model (Hunt et al. 1984). The model adequately simulated the population dynamics of bacteria and protozoa, and the flows of C, ^{14}C, N and ^{15}N in Soil B (clay loam). The model was then validated with data from Soil C (sandy loam) after making some minor changes to the values of the parameters to account for the reduced bacterial protection in this soil.

The model showed reductions of bacterial numbers and greater protozoa-induced mineralization of bacterial N that were consistent with the experimental data obtained in Chapter 5 and 6. The model provided additional information on the partitioning of recently incorporated C and N within organisms and various soil pools. Many of these pools could not be measured experimentally. Predation affected the partitioning of immobilized N differently depending on the texture of the soil. The model also showed that about 99% of the protozoa-induced N mineralization in the first 12 days of the simulation was due to direct excretion of NH_4-N by protozoa. The remaining 1% increase was due to increased bacterial NH_4-N mineralization.

Mathematical modelling of the microcosm experiment provided a rigorous link between soil organism populations and the processes in the C and N cycles. Differences in soil texture, which impacted on bacterial-protozoan interactions, were incorporated into the model to predict the effect that texture had on protozoan-induced mineralization of bacterial N.

The objective of Chapter 4 was to determine how manipulation of microbial activity in the field, through glucose addition during fertilizer N application, may affect the overall distribution and recovery of fertilizer N in a barley-soil system. It was thought that fertilizer immobilized into the microbial and non-microbial active N pools would remineralize at a rate sufficient to meet the demands of the barley crop. In this way these N pools would serve as a slow release fertilizer to help conserve fertilizer N in the plant-soil system, by reducing denitrification and leaching losses. Microbial activity due to glucose addition effectively reduced losses of fertilizer N by increased immobilization, however the remineralization of N did not occur at a rate sufficient to meet the demands of the barley crop. As a result, plant growth and plant uptake of N were less than in the glucose-minus controls. But, net N mineralization in the glucose-plus treatment was greater than in the glucose-minus control late in the season. Thus, it seems that an earlier and perhaps lower application rate of glucose may reduce N losses and still maintain adequate plant yields. Further research should be conducted in this area.

The soil biotic community consists of plants, bacteria, fungi, protozoa, mesofauna and macrofauna. Microbial and faunal populations play an important role in N mineralization and immobilization. The link between soil biota and soil and plant processes was explored in this thesis.

Future Research

This project has raised several points that could be the focus for future research. What factors affect the proportion of soil organic matter that is contained within the active and stabilized pools? Is it mainly a textural/mineralogical phenomenon or are other factors, such as chemical nature of the organic matter important as well? Does soil genesis play an important role in the nature of active organic matter, or are only the slower cycling stable and old phases influenced? Sanborn and Pawluk (1989) noted that much of organic matter in coarse-textured Black Chernozems coated the surfaces of sand grains, in contrast to the organic matter in fine-textured soils which is contained mainly within aggregates. This physical aspect would be expected to affect the accessibility of organic substrates to microbial populations, and also the accessibility of the microflora to faunal predators. Hendrix et al. (1986) said that differences in soil management may determine whether the detritus food web in soil is fungal dominated or bacterial dominated. If this is true one would expect the chemical nature of the soil organic matter to differ according to management since bacteria and fungi have different biochemical compositions (Juma and McGill 1986).

In Chapter 5 an approximate estimation of the habitable pore space available to protozoa and bacteria was calculated from water retention data using the capillary rise equation. It is difficult to make a mathematical link between these pore volume estimations and the impact that pore size distribution and tortuosity had on bacterial-protozoan interactions. Perhaps a more sophisticated procedure such as thin section fluorescence microscopy (Altemüller and van Vliet-Lanoe 1990) will aid in making the quantitative linkage between observable soil characteristics and microbial-faunal interactions, and the resulting effects on soil processes such as N mineralization.

Once this is done it may be possible to detemine an empirical or theoretical procedure for deriving the Kab parameter in the simulation model to account for the reduced protection of bacteria from protozoa in coarse-textured soil. In this thesis the Kab parameter used for the two soils was determined iteratively.

The simulation model developed in this thesis is by no means completely polished. It should be further tested on new experimental data and refined accordingly. It would be useful to test the model on soil that is not first sterilized. Ideally, the model would be expanded to include other soil organisms and it would accurately predict biological interactions and flows of C and N under varied environmental conditions such as in the field. According to Fiebleman (1972) the scientific method is completed when we can theoretically predict the outcome of natural phenomena and can control natural phenomena for practical applications. This may be more easily attainable in the non-biological sciences of physics and chemistry. It will be many years before we can confidently say that prediction and control of the complex soil ecosystem has been attained.

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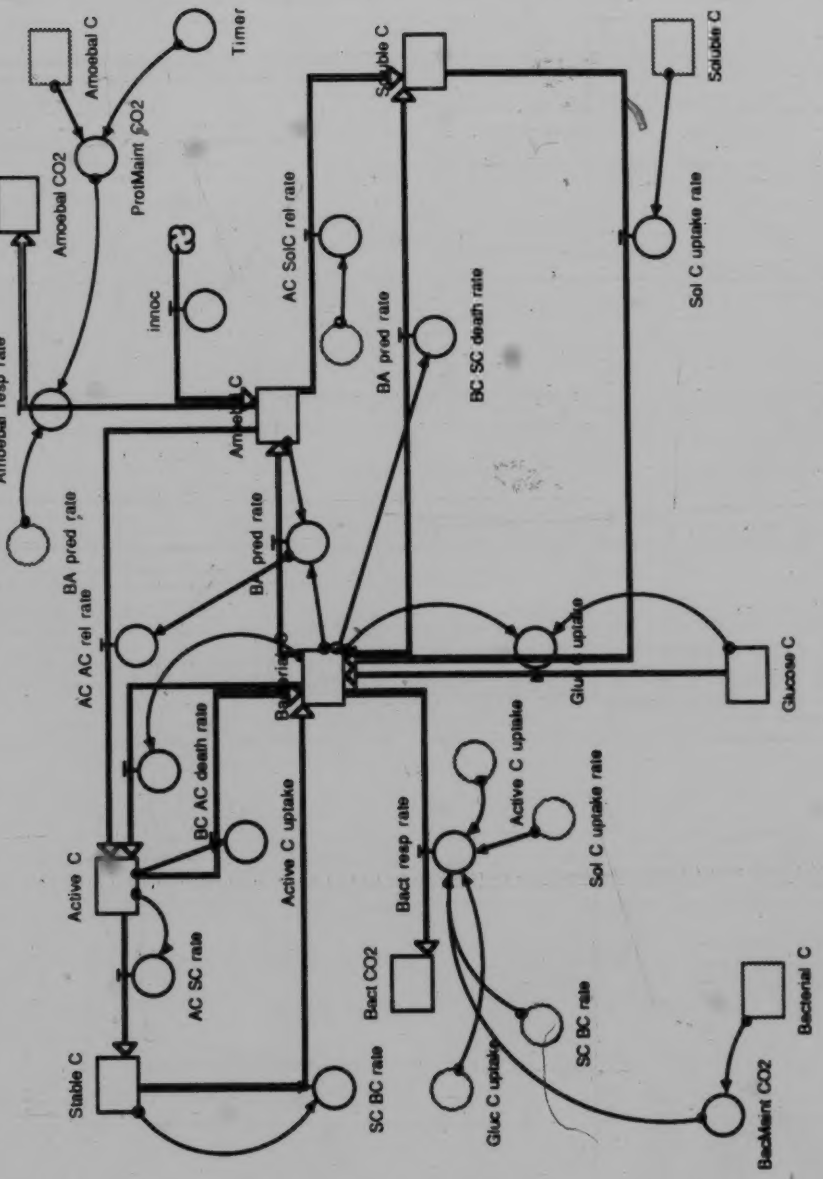
Appendix 1. Flow Diagrams for Simulation Model


Timer
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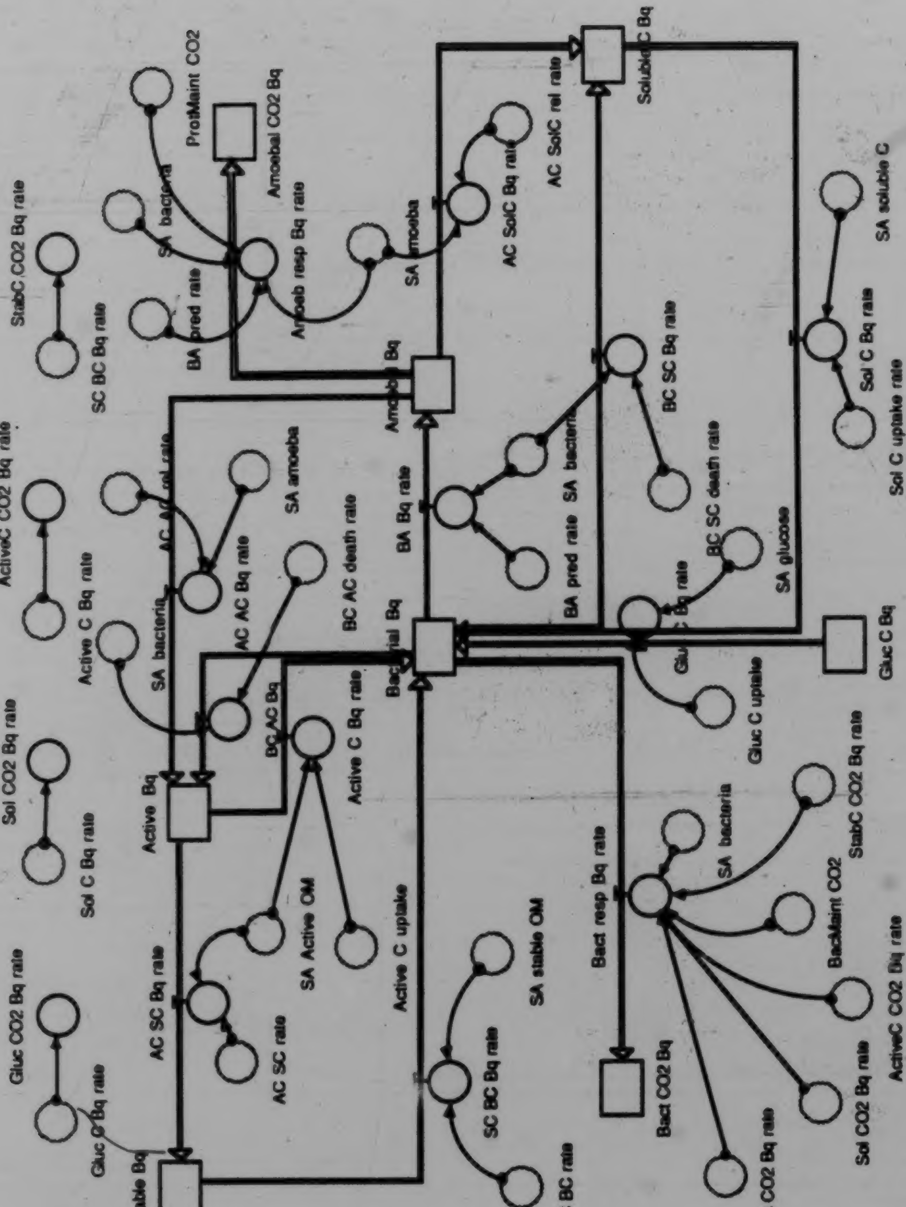
ProtMaint CO2
Amoebal CO2
Soluble C
Soluble C
AC SolC rel rate
Sol C uptake rate
innoc
BA pred rate
BC SC death rate
Amoebal resp rate
Amoebal C
BA pred rate
BA pred rate
AC AC rel rate
Gluc C uptake
Glucose C
Bacterial C
BC AC death rate
Active C uptake
Active C
Active C uptake
Bact resp rate
Sol C uptake rate
AC SC rate
Bact CO2
SC BC rate
Stable C
SC BC rate
Gluc C uptake
BacMaint CO2
Bacterial C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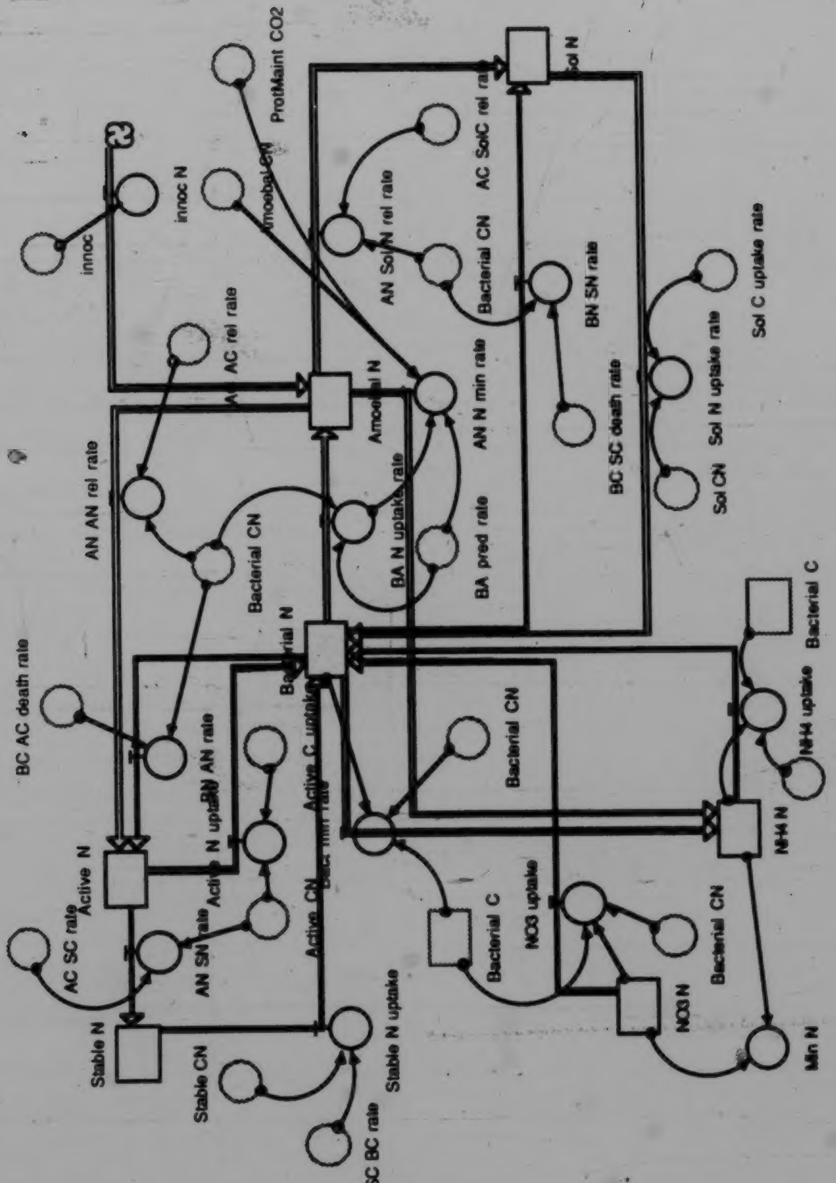
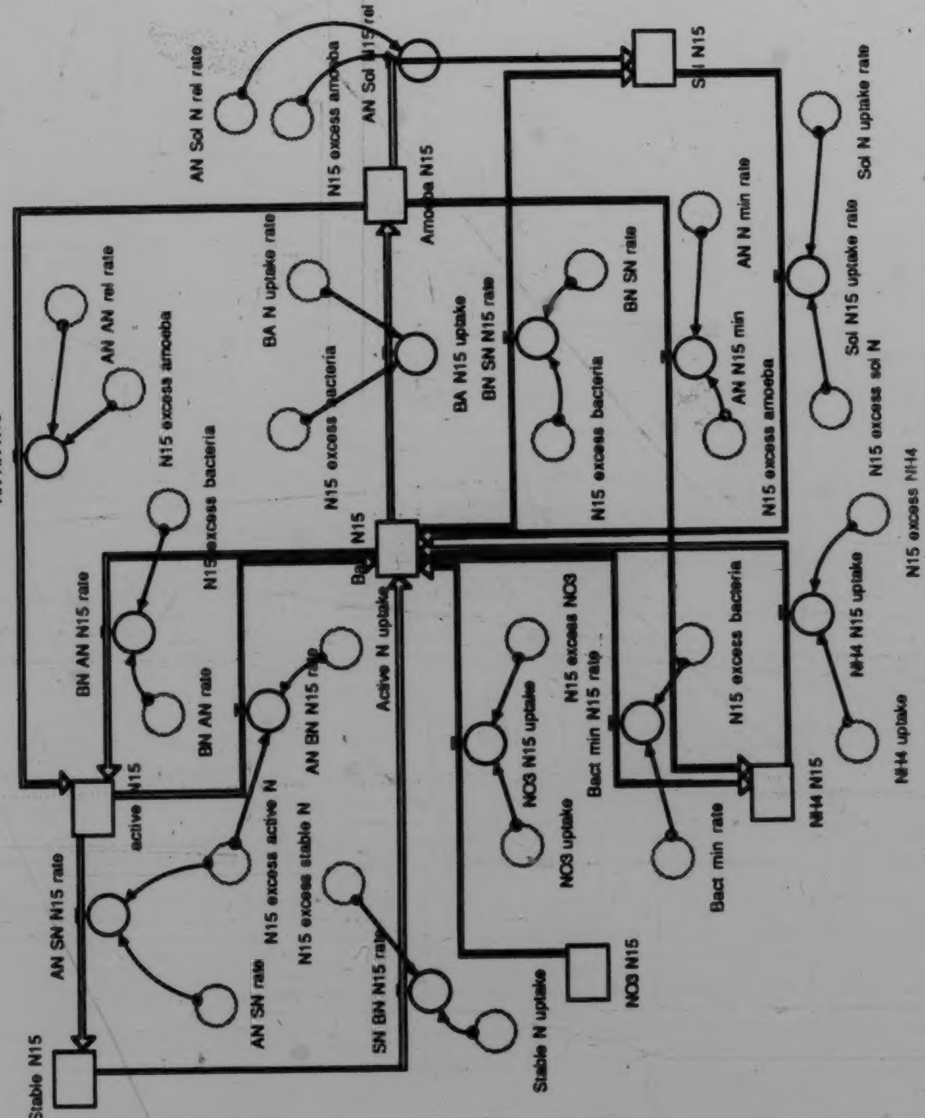
Gluc C Bq rate
Stable Bq
Sol CO2 Bq rate
Sol C Bq rate
ActiveC CO2 Bq rate
Active C Bq rate
StabC CO2 Bq rate
SC BC Bq rate
AC SC Bq rate
AC SC rate
Active Bq
SA Active OM
SA bacteria
Active C Bq rate
BC AC Bq
AC AC Bq rate
AC AC rel rate
SA amoeba
BC AC death rate
Active C uptake
SA stable OM
SC BC Bq rate
SC BC rate
Bacterial Bq
BA Bq rate
Amoeba Bq
BA pred rate
Amoeb resp Bq rate
SA bacteria
ProtMaint CO2
Amoebal CO2 Bq
SA amoeba
AC SolC Bq rate
AC SolC rel rate
Soluble C Bq
BC SC Bq rate
BA pred rate
SA bacteria
BC SC death rate
SA glucose
Gluc C Bq rate
Gluc C uptake
Gluc C Bq
Bact resp Bq rate
SA bacteria
BacMaint CO2
StabC CO2 Bq rate
ActiveC CO2 Bq rate
Sol CO2 Bq rate
Gluc CO2 Bq rate
Bact CO2 Bq
SA soluble C
Sol C Bq rate
Sol C uptake rate


BC AC death rate
AN AN rel rate
AN AC rel rate
innoc
innoc N
Amoebal CN
ProtMaint CO2
AC SC rate
Active N
AN SN rate
Active N uptake
BN AN rate
Active CN
Bact min rate
Active C uptake
Bacterial N
Bacterial CN
Amoebal N
BA N uptake rate
BA pred rate
AN N min rate
AN SolN rel rate
Bacterial CN
AC SolC rel rate
Sol N
BN SN rate
BC SC death rate
Sol CN
Sol N uptake rate
Sol C uptake rate
Stable N
Stable CN
Stable N uptake
SC BC rate
Bacterial C
NO3 uptake
Bacterial CN
NO3 N
Min N
Bacterial CN
NH4 N
NH4 uptake
Bacterial CN
Bacterial C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AN SN N15 rate
AN SN rate
active N15
N15 excess active N
N15 excess stable N
SN BN N15 rate
Stable N uptake
NO3 N15
BN AN N15 rate
BN AN rate
AN BN N15 rate
Active N uptake
Ba N15
N15 excess bacteria
AN AN N15
AN AN rel rate
N15 excess amoeba
N15 excess bacteria
BA N uptake rate
BA N15 uptake
BN SN N15 rate
Amoeba N15
AN Sol N rel rate
N15 excess amoeba
AN Sol N15 rel
NO3 N15 uptake
NO3 uptake
N15 excess NO3
Bact min N15 rate
Bact min rate
N15 excess bacteria
NH4 N15
NH4 N15 uptake
NH4 uptake
N15 excess NH4
N15 excess bacteria
BN SN rate
AN N15 min
AN N min rate
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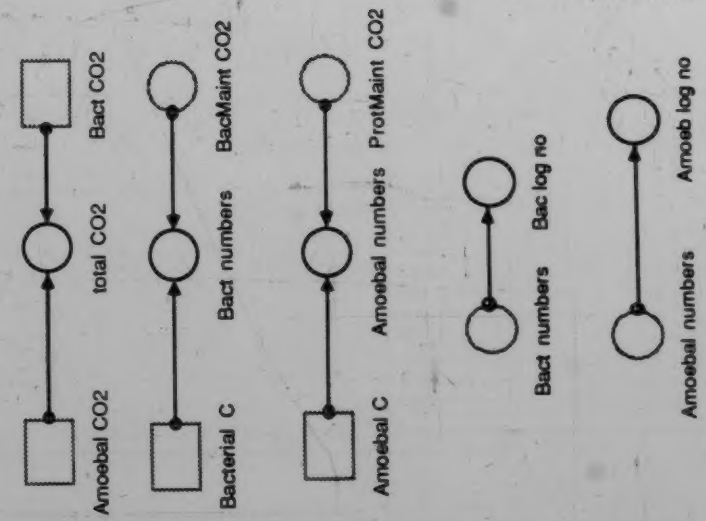
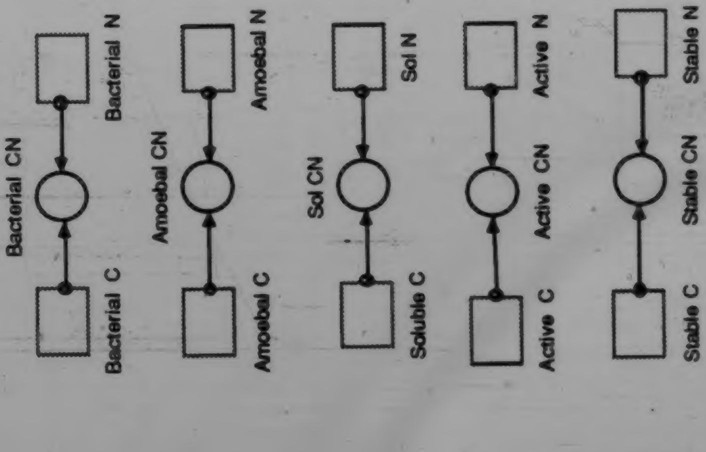
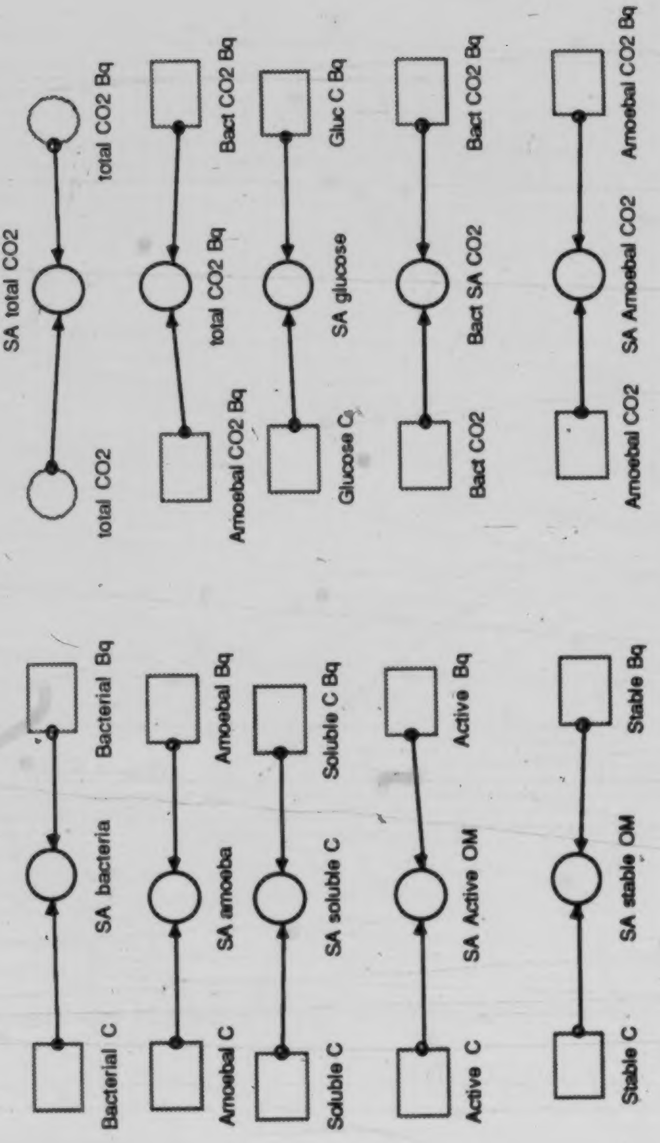
Sol N15
Sol N15 uptake rate
Sol N uptake rate
N15 excess sol N


Amoebal CO2
total CO2
Bact CO2
Bacterial C
Bact numbers
BacMaint CO2
Amoebal C
Amoebal numbers
ProtMaint CO2
Bact numbers
Bac log no
Amoebal numbers
Amoeb log no


Bacterial C
Bacterial CN
Bacterial N
Amoebal C
Amoebal CN
Amoebal N
Soluble C
Sol CN
Sol N
Active C
Active CN
Active N
Stable C
Stable CN
Stable N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Bacterial Bq
Amoebal C
SA amoeba
Amoebal Bq
Soluble C
SA soluble C
Soluble C Bq
Active C
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Active Bq
Stable C
SA stable OM
Stable Bq
total CO2
SA total CO2
total CO2 Bq
Amoebal CO2 Bq
total CO2 Bq
Bact CO2 Bq
Glucose C
SA glucose
Gluc C Bq
Bact CO2
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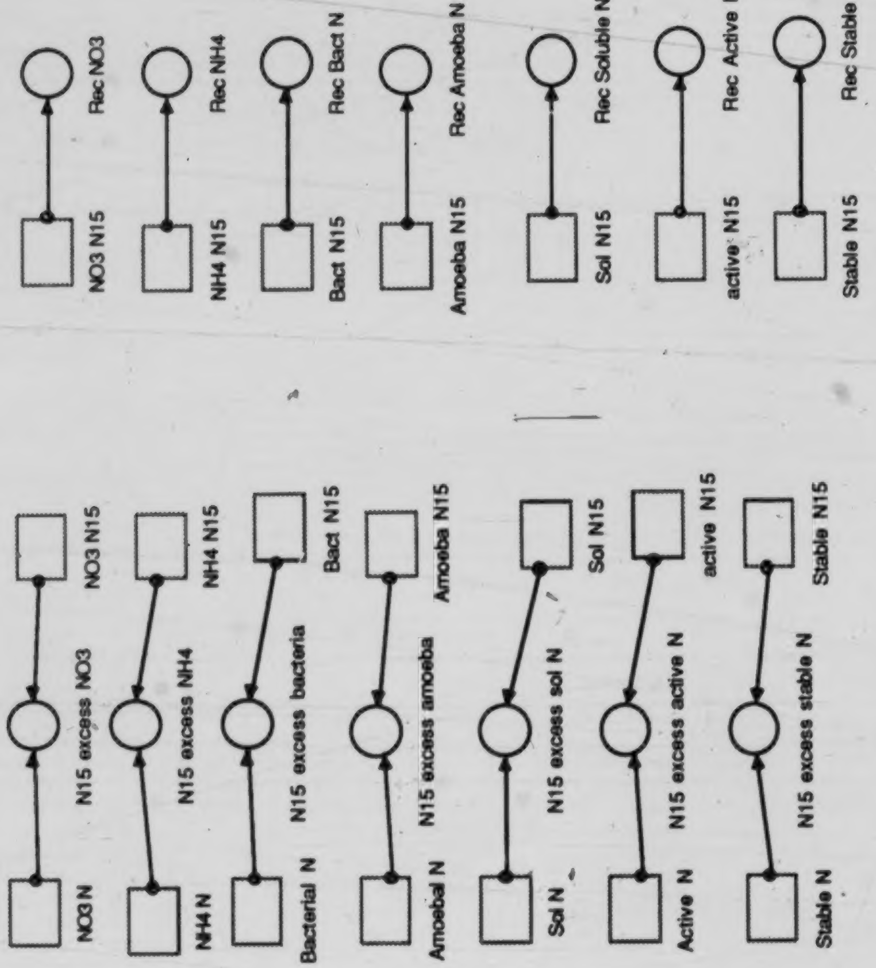
Amoebal CO2
SA Amoebal CO2
Amoebal CO2 Bq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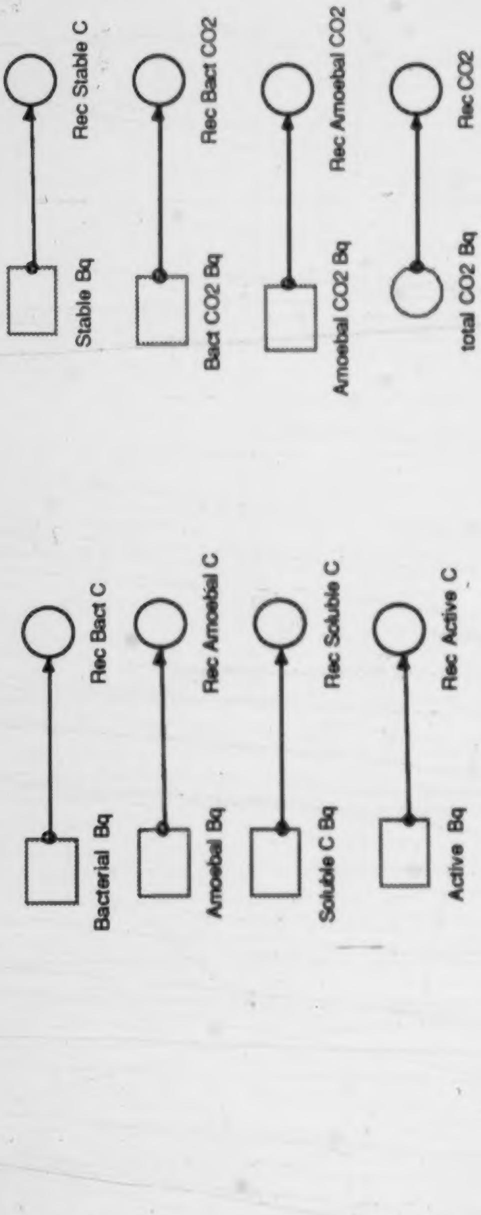
Rec NO3
NH4 N15
Rec NH4
Bact N15
Rec Bact N
Amoeba N15
Rec Amoeba N
Sol N15
Rec Soluble N
active N15
Rec Active N
Stable N15
Rec Stable N
NO3 N
N15 excess NO3
NO3 N15
NH4 N
N15 excess NH4
NH4 N15
Bacterial N
N15 excess bacteria
Bact N15
Amoebal N
N15 excess amoeba
Amoeba N15
Sol N
N15 excess sol N
Sol N15
Active N
N15 excess active N
active N15
Stable N
N15 excess stable N
Stable N15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Bact CO2 Bq
Rec Bact CO2
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Rec Amoebal CO2
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Rec CO2
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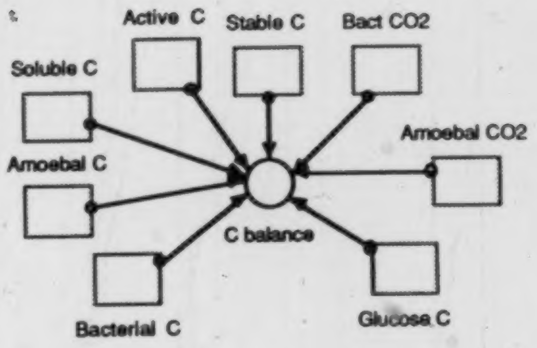
Rec Bact C
Amoebal Bq
Rec Amoebal C
Soluble C Bq
Rec Soluble C
Active Bq
Rec Active C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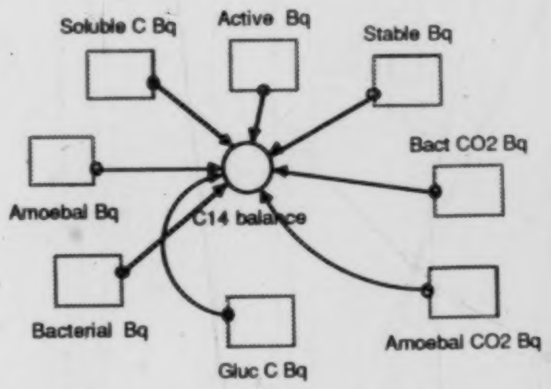
C balance
Bacterial C
Glucose C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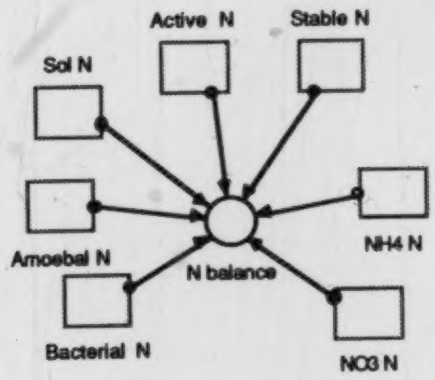
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Active N
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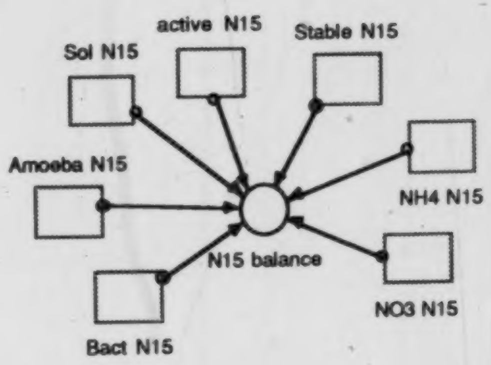
NH4 N
Amoebal N
N balance
Bacterial N
NO3 N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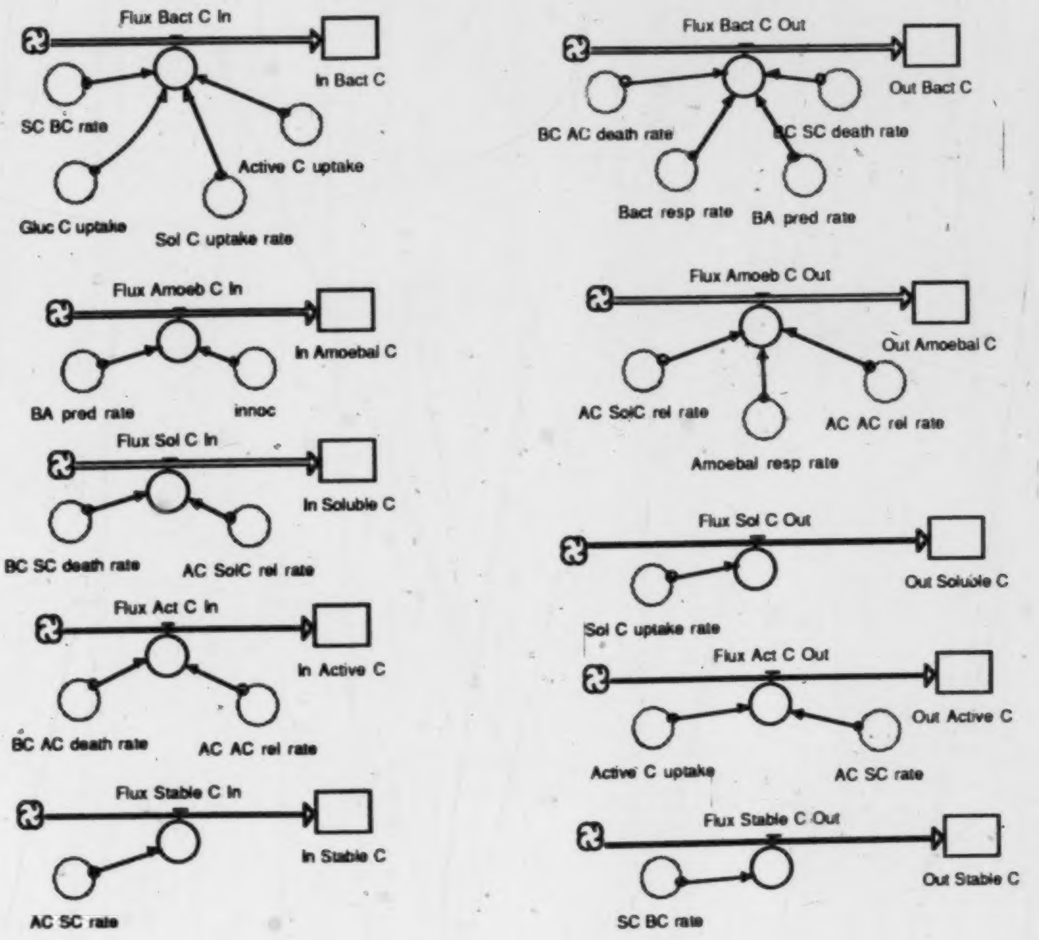
N15 balance
NO3 N15
Bact N15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In Bact C
SC BC rate
Active C uptake
Gluc C uptake
Sol C uptake rate
Flux Amoeb C In
In Amoebal C
BA pred rate
innoc
Flux Sol C In
In Soluble C
BC SC death rate
AC SolC rel rate
Flux Act C In
In Active C
BC AC death rate
AC AC rel rate
Flux Stable C In
In Stable C
AC SC rate
Flux Bact C Out
Out Bact C
BC AC death rate
BC SC death rate
Bact resp rate
BA pred rate
Flux Amoeb C Out
Out Amoebal C
AC SolC rel rate
AC AC rel rate
Amoebal resp rate
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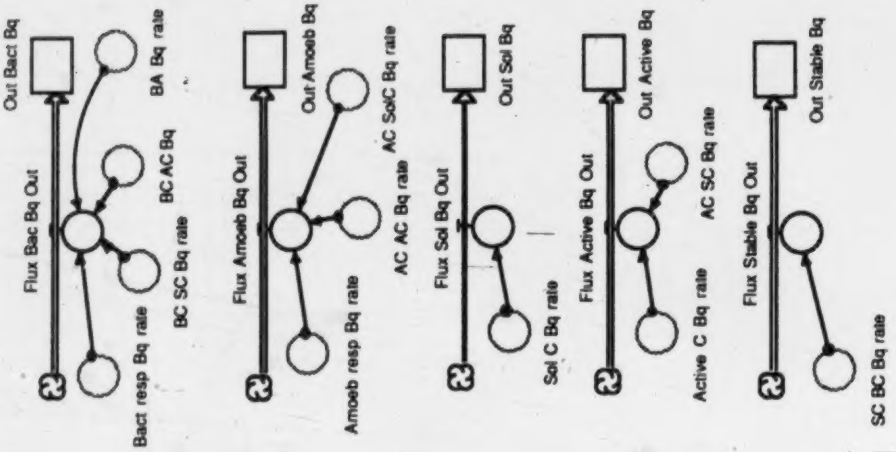
Out Stable C
SC BC rate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AC AC Bq rate
Amoeb resp Bq rate
Out Sol Bq
Flux Sol Bq Out
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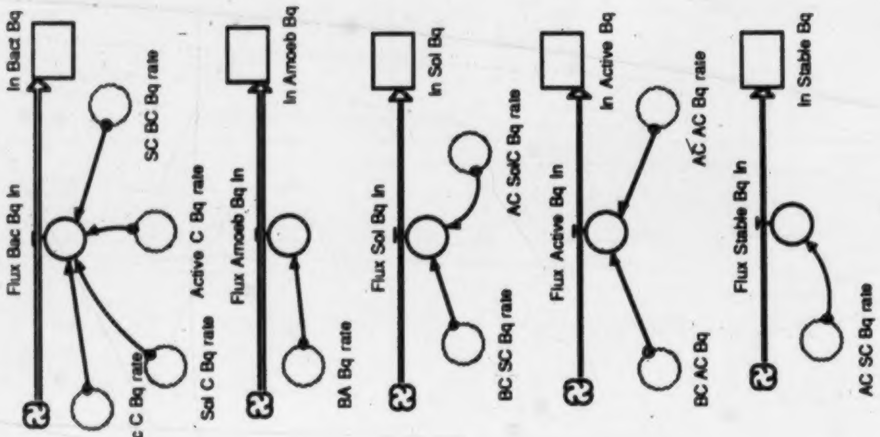
Active C Bq rate
Out Stable Bq
Flux Stable Bq Out
SC BC Bq rate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BA Bq rate
In Sol Bq
Flux Sol Bq In
AC SolC Bq rate
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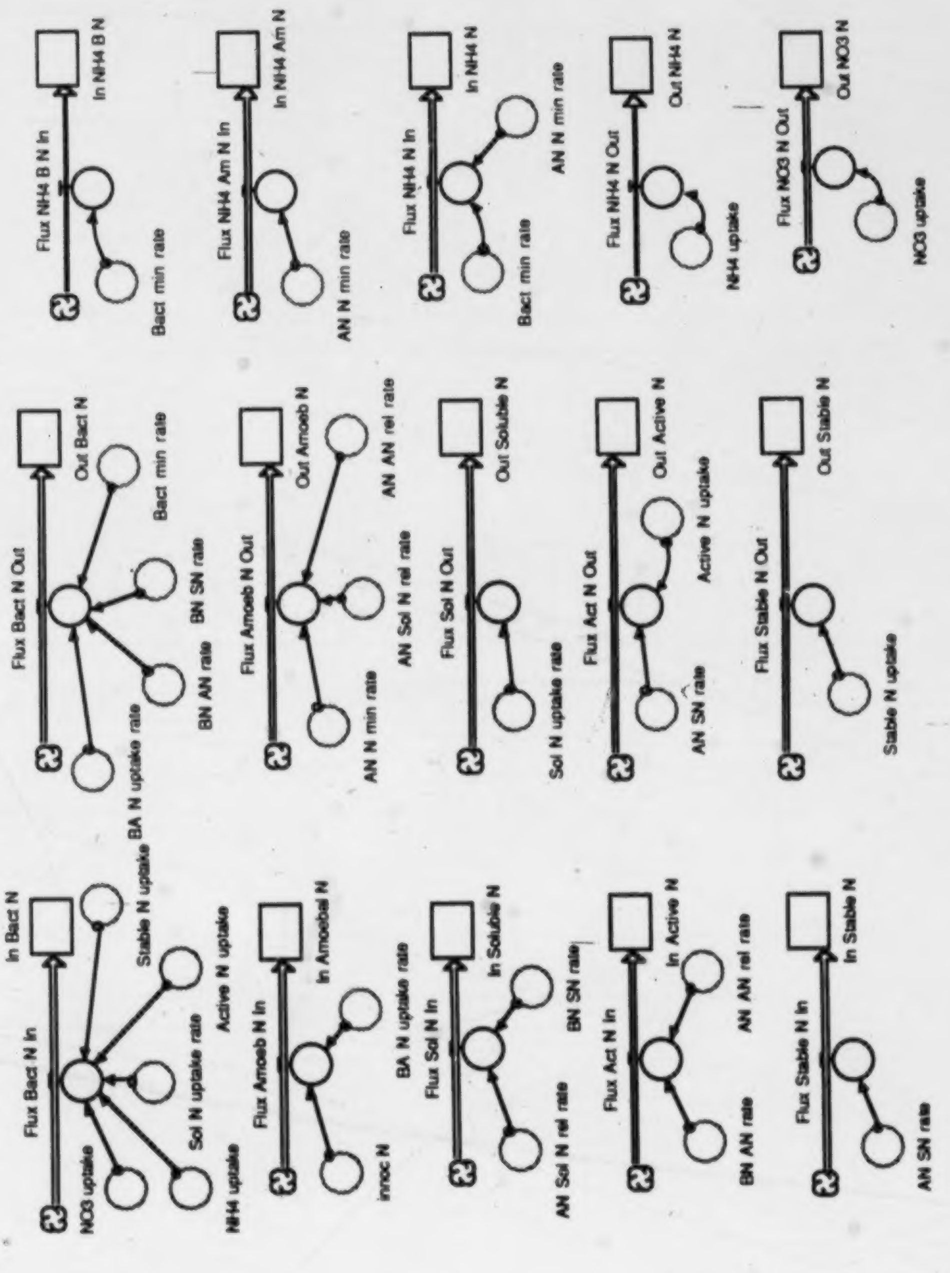
Flux Stable Bq In
AC SC Bq rate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Bact min rate
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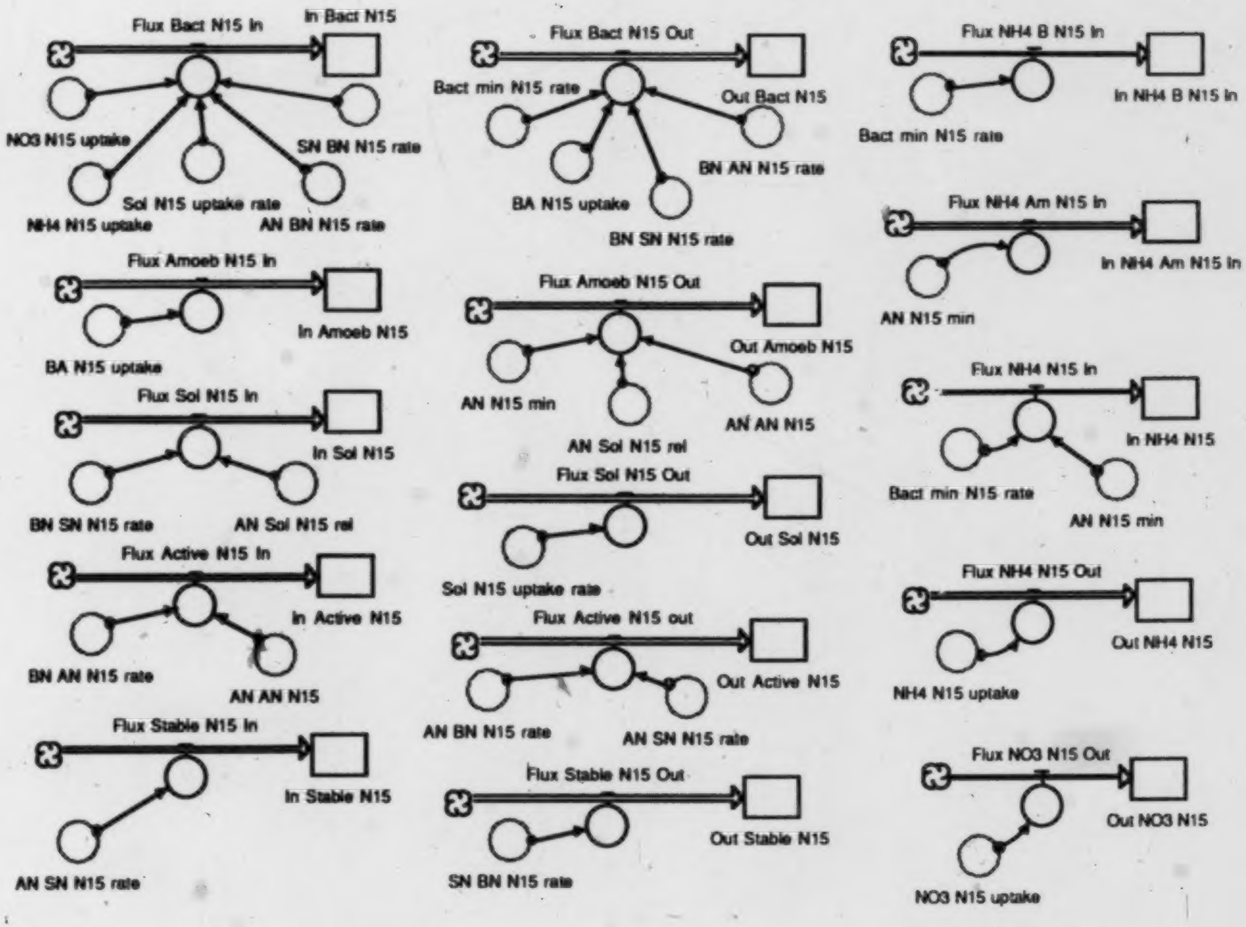
In NH4 Am N
AN N min rate
Flux NH4 N In
In NH4 N
Bact min rate
AN N min rate
Flux NH4 N Out
Out NH4 N
NH4 uptake
Flux NO3 N Out
Out NO3 N
NO3 uptake
Flux Bact N Out
Out Bact N
Bact min rate
BN SN rate
BN AN rate
BA N uptake rate
Flux Amoeb N Out
Out Amoeb N
AN AN rel rate
AN Sol N rel rate
AN N min rate
Flux Sol N Out
Out Soluble N
Sol N uptake rate
Flux Act N Out
Out Active N
Active N uptake
AN SN rate
Flux Stable N Out
Out Stable N
Stable N uptake
Flux Bact N In
In Bact N
NO3 uptake
Sol N uptake rate
Stable N uptake
Active N uptake
NH4 uptake
Flux Amoeb N In
In Amoebal N
BA N uptake rate
innoc N
Flux Sol N In
In Soluble N
BN SN rate
AN Sol N rel rate
Flux Act N In
In Active N
AN AN rel rate
BN AN rate
Flux Stable N In
In Stable N
AN SN rate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In Bact N15
NO3 N15 uptake
SN BN N15 rate
Sol N15 uptake rate
NH4 N15 uptake
AN BN N15 rate
Flux Amoeb N15 In
In Amoeb N15
BA N15 uptake
Flux Sol N15 In
In Sol N15
BN SN N15 rate
AN Sol N15 rel
Flux Active N15 In
In Active N15
BN AN N15 rate
AN AN N15
Flux Stable N15 In
In Stable N15
AN SN N15 rate
Flux Bact N15 Out
Bact min N15 rate
Out Bact N15
BN AN N15 rate
BA N15 uptake
BN SN N15 rate
Flux Amoeb N15 Out
Out Amoeb N15
AN N15 min
AN AN N15
AN Sol N15 rel
Flux Sol N15 Out
Out Sol N15
Sol N15 uptake rate
Flux Active N15 out
Out Active N15
AN BN N15 rate
AN SN N15 rate
Flux Stable N15 Out
Out Stable N15
SN BN N15 rate
Flux NH4 B N15 In
In NH4 B N15 In
Bact min N15 rate
Flux NH4 Am N15 In
In NH4 Am N15 In
AN N15 min
Flux NH4 N15 In
In NH4 N15
Bact min N15 rate
AN N15 min
Flux NH4 N15 Out
Out NH4 N15
NH4 N15 uptake
Flux NO3 N15 Out
Out NO3 N15
NO3 N15 uptake

Appendix 2. Equations for Simulation Model

Equations for the C Submodel

Active_C(t) = Active_C(t - dt) + (AC_AC_rel_rate + BC_AC_death_rate - Active_C_uptake - AC_SC_rate) * dt

INIT Active_C = 1308 {μg C/g soil}

INFLOWS:

AC_AC_rel_rate = BA_pred_rate * (1.0 - 0.60) {40% waste; 60% digestion} * 0.65 {fraction of the material which is insoluble or chemically stable}

BC_AC_death_rate = 0.075*Bacterial_C*0.65

OUTFLOWS:

Active_C_uptake = 0.25*0.0038*Active_C

{first order kinetics; k=0.00095 /day; Active C is pool size}

AC_SC_rate = 0.0005*Active_C {active C to stable C stablization rate}

Amoebal_C(t) = Amoebal_C(t - dt) + (BA_pred_rate + innoc - AC_SolC_rel_rate - Amoebal_resp_rate - AC_AC_rel_rate) * dt

INIT Amoebal_C = 0.00000000000001

INFLOWS:

BA_pred_rate = 3.6 {d} * Amoebal_C {μg C/g soil}

* (1/(1+ ((175 {μg C/g soil}/Bacterial_C {μg C/g soil})^10)))

innoc = Pulse (1.08,2.0,100)

OUTFLOWS:

AC_SolC_rel_rate = BA_pred_rate * (1.0 - 0.60) {40% waste; 60%digestion} * 0.35 {fraction of the material which is soluble and enters the active fraction}

Amoebal_resp_rate = BA_pred_rate*0.60 {%digestion}*(1-0.60) {production efficency} + ProtMaint_CO2 {maintenace coef./day}

AC_AC_rel_rate = BA_pred_rate * (1.0 - 0.60) {40% waste; 60% digestion} * 0.65 {fraction of the material which is insoluble or chemically stable}

Amoebal_CO2(t) = Amoebal_CO2(t - dt) + (Amoebal_resp_rate) * dt

INIT Amoebal_CO2 = 0.0000001

INFLOWS:

Amoebal_resp_rate = BA_pred_rate*0.60 {%digestion}*(1-0.60) {production efficency} + ProtMaint_CO2 {maintenace coef./day}

Bacterial_C(t) = Bacterial_C(t - dt) + (Active_C_uptake + Sol_C_uptake_rate + SC_BC_rate + Gluc_C_uptake_ - Bact_resp_rate - BA_pred_rate - BC_SC_death_rate - BC_AC_death_rate) * dt
INIT Bacterial_C = 111 {µg C/g soil}
INFLOWS:
Active_C_uptake = 0.25*0.0038*Active_C
{first order kinetics; k=0.00095 /day; Active C is pool size}
Sol_C_uptake_rate = 0.5*Soluble_C
{first order kinetics; k=0.5 /day; Soluble C is pool size}
SC_BC_rate = 0.0004*Stable_C {first order uptake}
Gluc_C_uptake_ = 7.2*Glucose_C*Bacterial_C/(580+Glucose_C)
OUTFLOWS:
Bact_resp_rate = (Gluc_C_uptake_*(1-0.525))+ (Sol_C_uptake_rate*(1-0.40)) + (Active_C_uptake*(1-0.40)) +(SC_BC_rate*(1-0.40)) +BacMaint_CO2
BA_pred_rate = 5*0.72 {d} * Amoebal_C {µg C/g soil}
* (1/(1+ ((175 {µg C/g soil}/Bacterial_C {µg C/g soil})^10)))

BC_SC_death_rate = 0.075*Bacterial_C*0.35
BC_AC_death_rate = 0.075*Bacterial_C*0.65

Bact_CO2(t) = Bact_CO2(t - dt) + (Bact_resp_rate) * dt
INIT Bact_CO2 = 0.0000001 {µg C/g soil}
INFLOWS:
Bact_resp_rate = (Gluc_C_uptake_*(1-0.525))+ (Sol_C_uptake_rate*(1-0.40)) + (Active_C_uptake*(1-0.40)) +(SC_BC_rate*(1-0.40)) +BacMaint_CO2

Glucose_C(t) = Glucose_C(t - dt) - (Gluc_C_uptake_) * dt
INIT Glucose_C = 360
OUTFLOWS:
Gluc_C_uptake_ = 7.2*Glucose_C*Bacterial_C/(580+Glucose_C)

Soluble_C(t) = Soluble_C(t - dt) + (AC_SolC_rel_rate + BC_SC_death_rate - Sol_C_uptake_rate) * dt
INIT Soluble_C = 80 {µg C/g soil}
INFLOWS:
AC_SolC_rel_rate = BA_pred_rate * (1.0 - 0.60) {40% waste; 60%digestion} * 0.35 {fraction of the material which is soluble and enters the active fraction}
BC_SC_death_rate = 0.075*Bacterial_C*0.35
OUTFLOWS:
Sol_C_uptake_rate = 0.5*Soluble_C
{first order kinetics; k=0.5 /day; Soluble C is pool size}

Stable_C(t) = Stable_C(t - dt) + (AC_SC_rate - SC_BC_rate) * dt
INIT Stable_C = 19855 {µg C/g soil}
INFLOWS:
AC_SC_rate = 0.0005*Active_C {active C to stable C stablization rate}
OUTFLOWS:
SC_BC_rate = 0.0004*Stable_C {first order uptake}

Equations for the ^{14}C Submodel

Active_Bq(t) = Active_Bq(t - dt) + (AC_AC_Bq_rate + BC_AC_Bq - Active_C_Bq_rate - AC_SC_Bq_rate) * dt
INIT Active_Bq = 0 {Bq/g soil}
INFLOWS:
AC_AC_Bq_rate = AC_AC_rel_rate*SA_amoeba
BC_AC_Bq = BC_AC_death_rate*SA_bacteria
OUTFLOWS:
Active_C_Bq_rate = Active_C_uptake*SA_Active_OM
AC_SC_Bq_rate = AC_SC_rate*SA_Active_OM

Amoebal_Bq(t) = Amoebal_Bq(t - dt) + (BA_Bq_rate - AC_SolC_Bq_rate - Amoeb_resp_Bq_rate - AC_AC_Bq_rate) * dt
INIT Amoebal_Bq = 0 {Bq/g soil}
INFLOWS:
BA_Bq_rate = BA_pred_rate*SA_bacteria

OUTFLOWS:
AC_SolC_Bq_rate = AC_SolC_rel_rate*SA_amoeba
Amoeb_resp_Bq_rate = (BA_pred_rate*0.60*(1-0.60)*SA_bacteria)+(ProtMaint_CO2*SA_amoeba)
AC_AC_Bq_rate = AC_AC_rel_rate*SA_amoeba

Amoebal_CO2_Bq(t) = Amoebal_CO2_Bq(t - dt) + (Amoeb_resp_Bq_rate) * dt
INIT Amoebal_CO2_Bq = 0 {Bq/g soil}
INFLOWS:
Amoeb_resp_Bq_rate = (BA_pred_rate*0.60*(1-0.60)*SA_bacteria)+(ProtMaint_CO2*SA_amoeba)

Bacterial_Bq(t) = Bacterial_Bq(t - dt) + (Active_C_Bq_rate + Sol_C_Bq_rate + SC_BC_Bq_rate + Gluc_C_Bq_rate - Bact_resp_Bq_rate - BA_Bq_rate - BC_SC_Bq_rate - BC_AC_Bq) * dt
INIT Bacterial_Bq = 0 {Bq/g soil}
INFLOWS:
Active_C_Bq_rate = Active_C_uptake*SA_Active_OM
Sol_C_Bq_rate = Sol_C_uptake_rate*SA_soluble_C
SC_BC_Bq_rate = SC_BC_rate*SA_stable_OM
Gluc_C_Bq_rate = Gluc_C_uptake_*SA_glucose
OUTFLOWS:
Bact_resp_Bq_rate = Gluc_CO2_Bq_rate+Sol_CO2_Bq_rate+ActiveC_CO2_Bq_rate+StabC_CO2_Bq_rate+(BacMaint_CO2*SA_bacteria)
BA_Bq_rate = BA_pred_rate*SA_bacteria
BC_SC_Bq_rate = BC_SC_death_rate*SA_bacteria
BC_AC_Bq = BC_AC_death_rate*SA_bacteria

Bact_CO2_Bq(t) = Bact_CO2_Bq(t - dt) + (Bact_resp_Bq_rate) * dt
INIT Bact_CO2_Bq = 0 {Bq/g soil}
INFLOWS:

Bact_resp_Bq_rate = Gluc_CO2_Bq_rate+Sol_CO2_Bq_rate+ActiveC_CO2_Bq_rate+StabC_CO2_Bq_rate+(BacMaint_CO2*SA_bacteria)

Gluc_C_Bq(t) = Gluc_C_Bq(t - dt) - (Gluc_C_Bq_rate) * dt
INIT Gluc_C_Bq = 360*13
OUTFLOWS:
Gluc_C_Bq_rate = Gluc_C_uptake_*SA_glucose

Soluble_C_Bq(t) = Soluble_C_Bq(t - dt) + (AC_SolC_Bq_rate + BC_SC_Bq_rate - Sol_C_Bq_rate) * dt
INIT Soluble_C_Bq = 0 {Bq/g soil}
INFLOWS:
AC_SolC_Bq_rate = AC_SolC_rel_rate*SA_amoeba
BC_SC_Bq_rate = BC_SC_death_rate*SA_bacteria
OUTFLOWS:
Sol_C_Bq_rate = Sol_C_uptake_rate*SA_soluble_C

Stable_Bq(t) = Stable_Bq(t - dt) + (AC_SC_Bq_rate - SC_BC_Bq_rate) * dt
INIT Stable_Bq = 0 {Bq/g soil}
INFLOWS:
AC_SC_Bq_rate = AC_SC_rate*SA_Active_OM
OUTFLOWS:
SC_BC_Bq_rate = SC_BC_rate*SA_stable_OM

Equations for the N Submodel

Active_N(t) = Active_N(t - dt) + (AN_AN_rel_rate + BN_AN_rate - Active_N_uptake - AN_SN_rate) * dt
INIT Active_N = Active_C/6.0
INFLOWS:
AN_AN_rel_rate = AC_AC_rel_rate/Bacterial_CN
BN_AN_rate = BC_AC_death_rate/Bacterial_CN
OUTFLOWS:

Active_N_uptake = Active_C_uptake/Active_CN
AN_SN_rate = AC_SC_rate/Active_CN

Amoebal_N(t) = Amoebal_N(t - dt) + (BA_N_uptake_rate + innoc_N - AN_Sol_N_rel_rate - AN_AN_rel_rate - AN_N_min_rate) * dt
INIT Amoebal_N = Amoebal_C/7
INFLOWS:
BA_N_uptake_rate = BA_pred_rate/Bacterial_CN
innoc_N = innoc/7
OUTFLOWS:
AN_Sol_N_rel_rate = AC_SolC_rel_rate/Bacterial_CN
AN_AN_rel_rate = AC_AC_rel_rate/Bacterial_CN
AN_N_min_rate = BA_N_uptake_rate*0.60 {%digestion} - 0.60*0.6*BA_pred_rate/7 + ProtMaint_CO2/Amoebal_CN

Bacterial_N(t) = Bacterial_N(t - dt) + (Active_N_uptake + Sol_N_uptake_rate + NH4_uptake + Stable_N_uptake + NO3_uptake - BA_N_uptake_rate - Bact_min_rate - BN_SN_rate - BN_AN_rate) * dt
INIT Bacterial_N = Bacterial_C/4.25
INFLOWS:
Active_N_uptake = Active_C_uptake/Active_CN
Sol_N_uptake_rate = Sol_C_uptake_rate/Sol_CN
NH4_uptake = IF (Bacterial_CN >4.5) THEN (2*1.68*Bacterial_C/(100 + NH4_N)) ELSE 0
Stable_N_uptake = SC_BC_rate/Stable_CN
NO3_uptake = IF (Bacterial_CN >4.5) THEN (4*1.68*Bacterial_C/(100 + NO3_N)) ELSE 0
OUTFLOWS:
BA_N_uptake_rate = BA_pred_rate/Bacterial_CN
Bact_min_rate = IF (Bacterial_CN < 4.25) THEN (Bacterial_N - (Bacterial_C/4.25)) ELSE 0
BN_SN_rate = BC_SC_death_rate/Bacterial_CN
BN_AN_rate = BC_AC_death_rate/Bacterial_CN

NH4_N(t) = NH4_N(t - dt) + (AN_N_min_rate + Bact_min_rate - NH4_uptake) * dt
INIT NH4_N = 33.5 {μg N/g soil}
INFLOWS:
AN_N_min_rate = BA_N_uptake_rate*0.60 {%digestion} - 0.60*0.6*BA_pred_rate/7 + ProtMaint_CO2/Amoebal_CN
Bact_min_rate = IF (Bacterial_CN < 4.25) THEN (Bacterial_N - (Bacterial_C/4.25)) ELSE 0
OUTFLOWS:
NH4_uptake = IF (Bacterial_CN >4.5) THEN (2*1.68*Bacterial_C/(100 + NH4_N)) ELSE 0

NO3_N(t) = NO3_N(t - dt) - (NO3_uptake) * dt
INIT NO3_N = 48.4 {μg N g soil}
OUTFLOWS:
NO3_uptake = IF (Bacterial_CN >4.5) THEN (4*1.68*Bacterial_C/(100 + NO3_N)) ELSE 0

Sol_N(t) = Sol_N(t - dt) + (AN_Sol_N_rel_rate + BN_SN_rate - Sol_N_uptake_rate) * dt
INIT Sol_N = Soluble_C/10.0
INFLOWS:
AN_Sol_N_rel_rate = AC_SolC_rel_rate/Bacterial_CN
BN_SN_rate = BC_SC_death_rate/Bacterial_CN
OUTFLOWS:
Sol_N_uptake_rate = Sol_C_uptake_rate/Sol_CN

Stable_N(t) = Stable_N(t - dt) + (AN_SN_rate - Stable_N_uptake) * dt
INIT Stable_N = 1805 {μg N g soil}
INFLOWS:
AN_SN_rate = AC_SC_rate/Active_CN
OUTFLOWS:
Stable_N_uptake = SC_BC_rate/Stable_CN

Equations for the ^{15}N Submodel

active_N15(t) = active_N15(t - dt) + (BN_AN_N15_rate + AN_AN_N15 - AN_SN_N15_rate - AN_BN_N15_rate) * dt
INIT active_N15 = 0.0 {N-15 excess}
INFLOWS:
BN_AN_N15_rate = BN_AN_rate*N15_excess_bacteria/100
AN_AN_N15 = AN_AN_rel_rate*N15_excess_amoeba/100
OUTFLOWS:
AN_SN_N15_rate = AN_SN_rate*N15_excess_active_N
AN_BN_N15_rate = Active_N_uptake*N15_excess_active_N/100

Amoeba_N15(t) = Amoeba_N15(t - dt) + (BA_N15_uptake - AN_AN_N15 - AN_Sol_N15_rel - AN_N15_min) * dt
INIT Amoeba_N15 = 0.0 {N-15 excess}
INFLOWS:
BA_N15_uptake = BA_N_uptake_rate*N15_excess_bacteria/100
OUTFLOWS:
AN_AN_N15 = AN_AN_rel_rate*N15_excess_amoeba/100
AN_Sol_N15_rel = AN_Sol_N_rel_rate*N15_excess_amoeba/100
AN_N15_min = AN_N_min_rate*N15_excess_amoeba/100

Bact_N15(t) = Bact_N15(t - dt) + (NO3_N15_uptake + Sol_N15_uptake_rate + AN_BN_N15_rate + SN_BN_N15_rate + NH4_N15_uptake - BN_SN_N15_rate - BN_AN_N15_rate - Bact_min_N15_rate - BA_N15_uptake) * dt
INIT Bact_N15 = 0.0 {μg N-15/g soil}
INFLOWS:
NO3_N15_uptake = NO3_uptake*N15_excess_NO3/100
Sol_N15_uptake_rate = Sol_N_uptake_rate*N15_excess_sol_N/100
AN_BN_N15_rate = Active_N_uptake*N15_excess_active_N/100
SN_BN_N15_rate = Stable_N_uptake*N15_excess_stable_N/100
NH4_N15_uptake = NH4_uptake*N15_excess_NH4/100
OUTFLOWS:
BN_SN_N15_rate = BN_SN_rate*N15_excess_bacteria/100

BN_AN_N15_rate = BN_AN_rate*N15_excess_bacteria/100
Bact_min_N15_rate = Bact_min_rate*N15_excess_bacteria/100
BA_N15_uptake = BA_N_uptake_rate*N15_excess_bacteria/100

NH4_N15(t) = NH4_N15(t - dt) + (Bact_min_N15_rate + AN_N15_min - NH4_N15_uptake) * dt
INIT NH4_N15 = 0.0 {N-15 excess}
INFLOWS:
Bact_min_N15_rate = Bact_min_rate*N15_excess_bacteria/100
AN_N15_min = AN_N_min_rate*N15_excess_amoeba/100
OUTFLOWS:
NH4_N15_uptake = NH4_uptake*N15_excess_NH4/100

NO3_N15(t) = NO3_N15(t - dt) - (NO3_N15_uptake) * dt
INIT NO3_N15 = 1.37 {μg N-15/g soil }
OUTFLOWS:
NO3_N15_uptake = NO3_uptake*N15_excess_NO3/100

Sol_N15(t) = Sol_N15(t - dt) + (BN_SN_N15_rate + AN_Sol_N15_rel - Sol_N15_uptake_rate) * dt
INIT Sol_N15 = 0.0{μg N-15/g soil }
INFLOWS:
BN_SN_N15_rate = BN_SN_rate*N15_excess_bacteria/100
AN_Sol_N15_rel = AN_Sol_N_rel_rate*N15_excess_amoeba/100
OUTFLOWS:
Sol_N15_uptake_rate = Sol_N_uptake_rate*N15_excess_sol_N/100

Stable_N15(t) = Stable_N15(t - dt) + (AN_SN_N15_rate - SN_BN_N15_rate) * dt
INIT Stable_N15 = 0.0 {N-15 excess}
INFLOWS:
AN_SN_N15_rate = AN_SN_rate*N15_excess_active_N
OUTFLOWS:
SN_BN_N15_rate = Stable_N_uptake*N15_excess_stable_N/100

Equations for Conversions

Active_CN = Active_C/Active_N
Amoebal_CN = Amoebal_C/Amoebal_N
Bacterial_CN = Bacterial_C/Bacterial_N
Sol_CN = Soluble_C/Sol_N
Stable_CN = Stable_C/Stable_N

Amoebal_numbers = (Amoebal_C+ProtMaint_CO2)/0.6e-3
Amoeb_log_no = LOG10(Amoebal_numbers)
ProtMaint_CO2 = IF (Timer>7) then (Amoebal_C*0.0025) else (Amoebal_C*0.025)
Timer = TIME
Bact_numbers = (Bacterial_C+(BacMaint_CO2{add maintenance}))/9.622e-8
Bac_log_no = LOG10(Bact_numbers)
BacMaint_CO2 = Bacterial_C*0.0025
total_CO2 = Bact_CO2+Amoebal_CO2
Min_N = NO3_N+NH4_N

SA_Active_OM = Active_Bq/Active_C
SA_amoeba = Amoebal_Bq/Amoebal_C
SA_Amoebal_CO2 = Amoebal_CO2_Bq/Amoebal_CO2
SA_bacteria = Bacterial_Bq/Bacterial_C
Bact_SA_CO2 = Bact_CO2_Bq/Bact_CO2
SA_glucose = Gluc_C_Bq/Glucose_C
SA_soluble_C = Soluble_C_Bq/Soluble_C
SA_stable_OM = Stable_Bq/Stable_C
SA_total_CO2 = total_CO2_Bq/total_CO2

N15_excess_active_N = (active_N15/Active_N)*100 {atom % excess}
N15_excess_amoeba = (Amoeba_N15/Amoebal_N)*100 {atom % excess}
N15_excess_bacteria = (Bact_N15/Bacterial_N)*100 {atom % excess}
N15_excess_NH4 = (NH4_N15/NH4_N)*100 {atom % excess}
N15_excess_NO3 = (NO3_N15/NO3_N)*100 {atom % excess}

```
N15_excess_sol_N = (Sol_N15/Sol_N) {atom % excess}*100
N15_excess_stable_N = (Stable_N15/Stable_N)*100 {atom % excess}

ActiveC_CO2_Bq_rate = Active_C_Bq_rate*(1-0.4)
Gluc_CO2_Bq_rate = Gluc_C_Bq_rate*(1-0.525)
Sol_CO2_Bq_rate = Sol_C_Bq_rate*(1-0.4)
StabC_CO2_Bq_rate = SC_BC_Bq_rate*(1-0.4)
total_CO2_Bq = Bact_CO2_Bq+Amoebal_CO2_Bq

Rec_Active_C = 100*Active_Bq/4680 {% rec. C-14}
Rec_Amoebal_C = 100*Amoebal_Bq/4680 {% rec. C-14}
Rec_Amoebal_CO2 = 100*Amoebal_CO2_Bq/4680 {% rec. C-14}
Rec_Bact_C = 100*Bacterial_Bq/4680 {% rec. C-14}
Rec_Bact_CO2 = 100*Bact_CO2_Bq/4680 {% rec. C-14}
Rec_CO2 = 100*total_CO2_Bq/4680 {% rec. C-14}
Rec_Soluble_C = 100*Soluble_C_Bq/4680 {% rec. C-14}
Rec_Stable_C = 100*Stable_Bq/4680 {% rec. C-14}

Rec_Active_N = (active_N15/1.37)*100 {% recovery of N-15}
Rec_Amoeba_N = (Amoeba_N15/1.37)*100 {% recovery of N-15}
Rec_Bact_N = (Bact_N15/1.37)*100 {% recovery of N-15}
Rec_NH4 = (NH4_N15/1.37)*100 {% recovery of N-15}
Rec_NO3 = (NO3_N15/1.37)*100 {% recovery of N-15}
Rec_Soluble_N = (Sol_N15/1.37)*100 {% recovery of N-15}
Rec_Stable_N = (Stable_N15/1.37)*100 {% recovery of N-15}

C_balance = Bacterial_C+Amoebal_C+Soluble_C+Active_C+Stable_C+
Amoebal_CO2 +Glucose_C+Bact_CO2
C14_balance = Bacterial_Bq+Amoebal_Bq+Soluble_C_Bq+Active_Bq+
Stable_Bq+Bact_CO2_Bq+Amoebal_CO2_Bq+Gluc_C_Bq
N_balance = Bacterial_N+Amoebal_N+Sol_N+Active_N+Stable_N+
NH4_N+NO3_N
N15_balance = Bact_N15+Amoeba_N15+Sol_N15+active_N15+
Stable_N15+NH4_N15+NO3_N15
```

Equations for Calculating Inflows and Outflows of C Pools

In_Active_C(t) = In_Active_C(t - dt) + (Flux_Act_C_In) * dt
INIT In_Active_C = 0
INFLOWS:
Flux_Act_C_In = BC_AC_death_rate+AC_AC_rel_rate

In_Amoebal_C(t) = In_Amoebal_C(t - dt) + (Flux_Amoeb_C_In) * dt
INIT In_Amoebal_C = 0
INFLOWS:
Flux_Amoeb_C_In = BA_pred_rate+innoc

In_Bact_C(t) = In_Bact_C(t - dt) + (Flux_Bact_C_In) * dt
INIT In_Bact_C = 0
INFLOWS:
Flux_Bact_C_In =
SC_BC_rate+Gluc_C_uptake_+Sol_C_uptake_rate+Active_C_uptake

In_Soluble_C(t) = In_Soluble_C(t - dt) + (Flux_Sol_C_In) * dt
INIT In_Soluble_C = 0
INFLOWS:
Flux_Sol_C_In = BC_SC_death_rate+AC_SolC_rel_rate

In_Stable_C(t) = In_Stable_C(t - dt) + (Flux_Stable_C_In) * dt
INIT In_Stable_C = 0
INFLOWS:
Flux_Stable_C_In = AC_SC_rate

Out_Active_C(t) = Out_Active_C(t - dt) + (Flux_Act_C_Out) * dt
INIT Out_Active_C = 0
INFLOWS:
Flux_Act_C_Out = Active_C_uptake+AC_SC_rate

Out_Amoebal_C(t) = Out_Amoebal_C(t - dt) + (Flux_Amoeb_C_Out) * dt

INIT Out_Amoebal_C = 0
INFLOWS:
Flux_Amoeb_C_Out = AC_SolC_rel_rate+Amoebal_resp_rate+AC_AC_rel_rate

Out_Bact_C(t) = Out_Bact_C(t - dt) + (Flux_Bact_C_Out) * dt
INIT Out_Bact_C = 0
INFLOWS:
Flux_Bact_C_Out =
BC_AC_death_rate+Bact_resp_rate+BA_pred_rate+BC_SC_death_rate

Out_Soluble_C(t) = Out_Soluble_C(t - dt) + (Flux_Sol_C_Out) * dt
INIT Out_Soluble_C = 0
INFLOWS:
Flux_Sol_C_Out = Sol_C_uptake_rate

Out_Stable_C(t) = Out_Stable_C(t - dt) + (Flux_Stable_C_Out) * dt
INIT Out_Stable_C = 0
INFLOWS:
Flux_Stable_C_Out = SC_BC_rate

Equations for Calculating Inflows and Outflows of ^{14}C Pools

In_Active_Bq(t) = In_Active_Bq(t - dt) + (Flux_Active_Bq_In) * dt
INIT In_Active_Bq = 0
INFLOWS:
Flux_Active_Bq_In = BC_AC_Bq+AC_AC_Bq_rate

In_Amoeb_Bq(t) = In_Amoeb_Bq(t - dt) + (Flux_Amoeb_Bq_In) * dt
INIT In_Amoeb_Bq = 0
INFLOWS:
Flux_Amoeb_Bq_In = BA_Bq_rate

In_Bact_Bq(t) = In_Bact_Bq(t - dt) + (Flux_Bac_Bq_In) * dt
INIT In_Bact_Bq = 0
INFLOWS:

Flux_Bac_Bq_In =
Gluc_C_Bq_rate+Sol_C_Bq_rate+Active_C_Bq_rate+SC_BC_Bq_rate

In_Sol_Bq(t) = In_Sol_Bq(t - dt) + (Flux_Sol_Bq_In) * dt
INIT In_Sol_Bq = 0
INFLOWS:
Flux_Sol_Bq_In = BC_SC_Bq_rate+AC_SolC_Bq_rate

In_Stable_Bq(t) = In_Stable_Bq(t - dt) + (Flux_Stable_Bq_In) * dt
INIT In_Stable_Bq = 0
INFLOWS:
Flux_Stable_Bq_In = AC_SC_Bq_rate

Out_Active_Bq(t) = Out_Active_Bq(t - dt) + (Flux_Active_Bq_Out) * dt
INIT Out_Active_Bq = 0
INFLOWS:
Flux_Active_Bq_Out = Active_C_Bq_rate+AC_SC_Bq_rate

Out_Amoeb_Bq(t) = Out_Amoeb_Bq(t - dt) + (Flux_Amoeb_Bq_Out) * dt
INIT Out_Amoeb_Bq = 0
INFLOWS:
Flux_Amoeb_Bq_Out =
Amoeb_resp_Bq_rate+AC_AC_Bq_rate+AC_SolC_Bq_rate

Out_Bact_Bq(t) = Out_Bact_Bq(t - dt) + (Flux_Bac_Bq_Out) * dt
INIT Out_Bact_Bq = 0
INFLOWS:
Flux_Bac_Bq_Out =
Bact_resp_Bq_rate+BC_SC_Bq_rate+BC_AC_Bq+BA_Bq_rate

Out_Sol_Bq(t) = Out_Sol_Bq(t - dt) + (Flux_Sol_Bq_Out) * dt
INIT Out_Sol_Bq = 0
INFLOWS:

Flux_Sol_Bq_Out = Sol_C_Bq_rate

Out_Stable_Bq(t) = Out_Stable_Bq(t - dt) + (Flux_Stable_Bq_Out) * dt

INIT Out_Stable_Bq = 0

INFLOWS:

Flux_Stable_Bq_Out = SC_BC_Bq_rate

Equations for Calculating Inflows and Outflows of N Pools

In_Active_N(t) = In_Active_N(t - dt) + (Flux_Act_N_In) * dt

INIT In_Active_N = 0

INFLOWS:

Flux_Act_N_In = BN_AN_rate+AN_AN_rel_rate

In_Amoebal_N(t) = In_Amoebal_N(t - dt) + (Flux_Amoeb_N_In) * dt

INIT In_Amoebal_N = 0

INFLOWS:

Flux_Amoeb_N_In = innoc_N+BA_N_uptake_rate

In_Bact_N(t) = In_Bact_N(t - dt) + (Flux_Bact_N_In) * dt

INIT In_Bact_N = 0

INFLOWS:

Flux_Bact_N_In = NO3_uptake+NH4_uptake+Sol_N_uptake_rate+Active_N_uptake+Stable_N_uptake

In_NH4_Am_N(t) = In_NH4_Am_N(t - dt) + (Flux_NH4_Am_N_In) * dt

INIT In_NH4_Am_N = 0

INFLOWS:

Flux_NH4_Am_N_In = AN_N_min_rate

In_NH4_B_N(t) = In_NH4_B_N(t - dt) + (Flux_NH4_B_N_In) * dt

```
INIT In_NH4_B_N = 0
INFLOWS:
Flux_NH4_B_N_In = Bact_min_rate

In_NH4_N(t) = In_NH4_N(t - dt) + (Flux_NH4_N_In) * dt
INIT In_NH4_N = 0
INFLOWS:
Flux_NH4_N_In = Bact_min_rate+AN_N_min_rate

In_Soluble_N(t) = In_Soluble_N(t - dt) + (Flux_Sol_N_In) * dt
INIT In_Soluble_N = 0
INFLOWS:
Flux_Sol_N_In = AN_Sol_N_rel_rate+BN_SN_rate

In_Stable_N(t) = In_Stable_N(t - dt) + (Flux_Stable_N_In) * dt
INIT In_Stable_N = 0
INFLOWS:
Flux_Stable_N_In = AN_SN_rate

Out_Active_N(t) = Out_Active_N(t - dt) + (Flux_Act_N_Out) * dt
INIT Out_Active_N = 0
INFLOWS:
Flux_Act_N_Out = AN_SN_rate+Active_N_uptake

Out_Amoeb_N(t) = Out_Amoeb_N(t - dt) + (Flux_Amoeb_N_Out) *
dt
INIT Out_Amoeb_N = 0
INFLOWS:
Flux_Amoeb_N_Out = AN_N_min_rate+AN_Sol_N_rel_rate+AN_AN_rel_rate

Out_Bact_N(t) = Out_Bact_N(t - dt) + (Flux_Bact_N_Out) * dt
INIT Out_Bact_N = 0
INFLOWS:
Flux_Bact_N_Out =
BA_N_uptake_rate+BN_AN_rate+BN_SN_rate+Bact_min_rate
```

Out_NH4_N(t) = Out_NH4_N(t - dt) + (Flux_NH4_N_Out) * dt
INIT Out_NH4_N = 0
INFLOWS:
Flux_NH4_N_Out = NH4_uptake

Out_NO3_N(t) = Out_NO3_N(t - dt) + (Flux_NO3_N_Out) * dt
INIT Out_NO3_N = 0
INFLOWS:
Flux_NO3_N_Out = NO3_uptake

Out_Soluble_N(t) = Out_Soluble_N(t - dt) + (Flux_Sol_N_Out) * dt
INIT Out_Soluble_N = 0
INFLOWS:
Flux_Sol_N_Out = Sol_N_uptake_rate

Out_Stable_N(t) = Out_Stable_N(t - dt) + (Flux_Stable_N_Out) * dt
INIT Out_Stable_N = 0
INFLOWS:
Flux_Stable_N_Out = Stable_N_uptake

Equations for Calculating Inflows and Outflows of ^{15}N Pools

In_Active_N15(t) = In_Active_N15(t - dt) + (Flux_Active_N15_In) * dt
INIT In_Active_N15 = 0
INFLOWS:
Flux_Active_N15_In = BN_AN_N15_rate+AN_AN_N15

In_Amoeb_N15(t) = In_Amoeb_N15(t - dt) + (Flux_Amoeb_N15_In) * dt
INIT In_Amoeb_N15 = 0
INFLOWS:
Flux_Amoeb_N15_In = BA_N15_uptake

In_Bact_N15(t) = In_Bact_N15(t - dt) + (Flux_Bact_N15_In) * dt
INIT In_Bact_N15 = 0
INFLOWS:
Flux_Bact_N15_In = NO3_N15_uptake+NH4_N15_uptake+Sol_N15_uptake_rate+AN_BN_N15_rate+SN_BN_N15_rate

In_NH4_Am_N15_In(t) = In_NH4_Am_N15_In(t - dt) + (Flux_NH4_Am_N15_In) * dt
INIT In_NH4_Am_N15_In = 0
INFLOWS:
Flux_NH4_Am_N14_In = AN_N15_min

In_NH4_B_N15_In(t) = In_NH4_B_N15_In(t - dt) + (Flux_NH4_B_N15_In) * dt
INIT In_NH4_B_N15_In = 0
INFLOWS:
Flux_NH4_B_N15_In = Bact_min_N15_rate

In_NH4_N15(t) = In_NH4_N15(t - dt) + (Flux_NH4_N15_In) * dt
INIT In_NH4_N15 = 0
INFLOWS:
Flux_NH4_N15_In = Bact_min_N15_rate+AN_N15_min

In_Sol_N15(t) = In_Sol_N15(t - dt) + (Flux_Sol_N15_In) * dt
INIT In_Sol_N15 = 0
INFLOWS:
Flux_Sol_N15_In = BN_SN_N15_rate+AN_Sol_N15_rel

In_Stable_N15(t) = In_Stable_N15(t - dt) + (Flux_Stable_N15_In) * dt
INIT In_Stable_N15 = 0
INFLOWS:
Flux_Stable_N15_In = AN_SN_N15_rate

Out_Active_N15(t) = Out_Active_N15(t - dt) + (Flux_Active_N15_out) * dt
INIT Out_Active_N15 = 0
INFLOWS:
Flux_Active_N15_out = AN_BN_N15_rate+AN_SN_N15_rate

Out_Amoeb_N15(t) = Out_Amoeb_N15(t - dt) + (Flux_Amoeb_N15_Out) * dt
INIT Out_Amoeb_N15 = 0
INFLOWS:
Flux_Amoeb_N15_Out = AN_N15_min+AN_Sol_N15_rel+AN_AN_N15

Out_Bact_N15(t) = Out_Bact_N15(t - dt) + (Flux_Bact_N15_Out) * dt
INIT Out_Bact_N15 = 0
INFLOWS:
Flux_Bact_N15_Out = Bact_min_N15_rate+BA_N15_uptake+BN_SN_N15_rate+BN_AN_N15_rate

Out_NH4_N15(t) = Out_NH4_N15(t - dt) + (Flux_NH4_N15_Out) * dt
INIT Out_NH4_N15 = 0
INFLOWS:
Flux_NH4_N15_Out = NH4_N15_uptake

Out_NO3_N15(t) = Out_NO3_N15(t - dt) + (Flux_NO3_N15_Out) * dt
INIT Out_NO3_N15 = 0
INFLOWS:
Flux_NO3_N15_Out = NO3_N15_uptake

Out_Sol_N15(t) = Out_Sol_N15(t - dt) + (Flux_Sol_N15_Out) * dt
INIT Out_Sol_N15 = 0

INFLOWS:
Flux_Sol_N15_Out = Sol_N15_uptake_rate

Out_Stable_N15(t) = Out_Stable_N15(t - dt) + (Flux_Stable_N15_Out) * dt
INIT Out_Stabe_N15 = 0
INFLOWS:
Flux_Stable_N15_Out = SN_BN_N15_rate